JOURNAL OF AGRICULTURAL RESEARCH

VOL. 61 WASHINGTON, D. C., JULY 1, 1940 No. 1

INVERSION OF SUCROSE IN THE DIFFERENT PARTS OF THE SUGARCANE STALK [1]

By J. I. LAURITZEN, *senior physiologist, Division of Sugar Plant Investigations, Bureau of Plant Industry,* and R. T. BALCH, *senior chemist, Carbohydrate Research Division, Bureau of Agricultural Chemistry and Engineering, United States Department of Agriculture* [2]

INTRODUCTION

As sugarcane (*Saccharum* spp.) matures, the sucrose content tends to become more or less equal throughout the millable portion of the stalk. In Louisiana, as in many Temperate Zone and some Torrid Zone sugar-producing countries, sugarcane never reaches maturity. If the stalk, as it is prepared for the mill,[3] is sectioned into three equal lengths and each section analyzed separately, it will be found that there is a considerable gradient in sucrose content from the top to the middle to the bottom section, the smallest amount being in the top third and the largest in the bottom third. As a rule, the difference is greater between the top and middle thirds than between the middle and bottom thirds. This gradient varies with the variety and the maturity of the cane. There also is an obvious difference in the vegetative condition of the tissues in these three parts of the cane stalk, particularly between the top and the bottom thirds, the middle third resembling more closely the bottom section. The top third is greener, softer, and more active physiologically than the other two sections.

The object of the investigation reported herein was to determine what bearing, if any, this difference in sucrose content and vegetative condition has on inversion of sucrose in harvested sugarcane.

REVIEW OF LITERATURE

Coates (*3*) reported more inversion of sucrose in the top third than in the bottom third of the stalk of certain varieties of sugarcane (P. O. J. 36 and P. O. J. 234) when whole stalks were stored in the shade and sectioned and analyzed after different periods of time. Cross and Harris (*4*), working in Argentina, indicated that in the varieties P. O. J. 36, P. O. J. 213, P. O. J. 234, P. O. J. 139, Kavangire, and Criolla the upper half of the stalk had a smaller sucrose content than the lower half. When samples of the upper and lower halves of 10 stalks of these varieties were stored for 10 days under comparable conditions, the percentage of inversion was greater in the upper half than in the lower half in all varieties except Criolla. In the case of Criolla the

[1] Received for publication April 19, 1940.

[2] For some of the Brix analyses and the apparent sucrose analyses the writers are indebted to R. B. Bisland and D. D. Sullivant, both formerly of the Division of Sugar Plant Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] In the preparation of sugarcane for the mill the stalk is cut off at the ground level, stripped of its leaves, and topped at a point as near the terminal bud as is justifiable from the standpoint of sucrose content. Change in color and appearance of the stalk has been used as an indication of this point. This point, or more appropriately region, moves up the stalk as the cane matures. Recently the point of topping has been influenced by the manner of maturing in different varieties (*2*).[4] In connection with the experimental work reported in this paper, the point of cutting was made as uniform as practicable.

[4] Italic numbers in parentheses refer to Literature Cited, p. 16.

inversion was greater in the lower half of the stalk than in the upper half in two out of three experiments. In all varieties the weight loss during storage was greater in the upper half of the stalk than in the lower half. Cross and Harris state that inversion was due to invertase present in the cane and that the concentration of invertase was relatively higher in the upper part of the stalk than in the lower part.

MATERIAL AND METHODS

Five experiments were conducted at Houma, La., during 4 years, 1932, 1933, 1934, and 1936, in which whole-stalk samples and one-third-stalk samples from the same lots of cane were placed in storage. In the first two experiments the samples were stored under controlled conditions of temperature and humidity, and in the last three experiments they were stored in the shade in an open shed. In the first two experiments sectioned and whole-stalk samples were stored in separate piles on a rack, the stalks lying parallel with all the cut ends exposed to the air; in the last three the samples were stored one layer thick on a rack, one whole-stalk sample alternating with the sections of another sample, the ends of the sections being separated by 4 to 6 inches. This latter arrangement was adopted in order to insure a more uniform evaporation from all samples than obtained in samples stored under controlled storage conditions.

Shade was provided by means of a shed 20 feet wide, 50 feet long, and 8 feet high, facing north, open in the front, and with provision for opening the lower 4 feet of the back.

Constant temperature was maintained in insulated rooms 10 feet wide, 12 feet long, and 9.5 feet high by means of refrigeration and electrical heating units thermoelectrically controlled. Humidity control was obtained by using water humidifiers and calcium chloride dryers.

The variety P. O. J. 36–M was used in four experiments and Co. 290 in one.

In a given experiment a quantity of sugarcane from a plot of uniform stand was harvested and thoroughly mixed as it was placed in a long pile, the stalks lying parallel with the butts in one direction. Samples consisting of 30 stalks each were then selected by drawing stalks at random from the pile. Both ends of each stalk were trimmed so that the cuts were at right angles to the length of the stalk. These 30-stalk samples were divided into two lots. The stalks of one lot were cut into three equal lengths (each whole stalk forming three short lengths), while the other lot was held as whole-stalk samples. Check samples were analyzed immediately, and the remaining sectioned and whole-stalk samples were placed in storage. After different periods of storage whole-stalk samples were cut into three equal lengths and analyzed along with samples sectioned before storage. In the last experiment conducted (with Co. 290), whole-stalk samples were analyzed at the various periods of storage. Five 30-stalk samples divided into sections, as well as five of the whole-stalk samples, were analyzed for each period of storage, and the results in each case are averages of five separate analyses.

The methods of analysis were the same as those employed in earlier work (*6*).

The sugar yields were calculated by means of the Winter-Carp-Geerligs formula (*5*), the simplified method developed by Arceneaux (*1*) being used. The yields were corrected for loss in weight of stored cane. Varietal milling factors were not used in connection with any of the calculated yields.

In certain instances, the yields of sucrose per ton of cane for the whole-stalk sample were calculated from data obtained from the sectioned sample. The yields were first calculated for the individual sectioned sample, corrected for weight loss in case of stored samples, and multiplied by a ratio factor obtained by dividing the original weight of the sectioned sample by the original weight of the whole-stalk sample. The yields thus obtained from the three sections of a particular sample were added to obtain the yield for the whole-stalk sample. In each of the experiments the ratio factors were obtained from the sectioned check samples and the samples sectioned before storage. The ratios of the top-, middle-, and bottom-section weights to the weight of the whole-stalk sample, expressed as percentages, were very constant in the samples of a given experiment and fairly constant from experiment to experiment (table 1).

TABLE 1.—*Average weights of whole-stalk samples, the ratio factors of sectioned samples, and the standard errors of these factors*

[The weights and ratios are the averages of the sectioned check samples and those sectioned before storage in each of 5 experiments at the time they were initiated]

Variety	Date experiment started	Samples used in calculations	Average weight of whole-stalk samples	Ratio of weight of sections to weight of whole stalks		
				Top	Middle	Bottom
	1932	*Number*	*Pounds*	*Percent*	*Percent*	*Percent*
P. O. J. 36–M	Dec. 8	25	46.53	28.97±.28	33.57±.08	37.46±.36
	1933					
Do	Nov. 8	40	48.94	28.86±.23	33.35±.12	37.79±.25
	1934					
Do	Nov. 2	25	48.19	28.83±.23	34.39±.09	36.76±.16
Do	Nov. 28	15	52.90	30.59±.51	33.44±.40	35.97±.19
	1936					
Co. 290	Oct. 30	20	71.70	29.70±.15	33.18±.09	37.13±.19

These same factors were used to calculate the probable original weights of sectioned samples of cane sectioned after storage. The initial weight of the whole-stalk sample and the weights of the sample sectioned after storage being known, it was possible to calculate the percentage loss of weight in the top-, middle-, and bottom-third sections of the whole-stalk sample.

The inverse correlation between the percentage of increase in Brix and the percentage of loss in weight during storage was calculated for the top and bottom portions of cane sectioned before storage, the data obtained from all five experiments being used. The correlation coefficient and standard error for the top sections were 0.949 and 0.033 and for the bottom sections 0.896 and 0.063, respectively. The percentage of increase in Brix, of course, is not equivalent to the percentage of loss in weight, but it definitely indicates the trends in loss in weight.

In certain instances the percentage of loss of sucrose in juice was calculated. In obtaining these values the apparent sucrose in juice from stored samples was corrected for weight loss before the percentage of loss was determined.

The statistical calculations given in tables 2 and 3 are based on the analysis of variance. The values given represent the difference in purity required for significance when $P=0.05$ and when $P=0.01$; in other words, the differences given may be expected to occur by chance alone 5 times in 100 when $P=0.05$ and 1 time in 100 when $P=0.01$.

EXPERIMENTAL DATA

Results obtained in connection with other experiments show that the loss in weight is primarily due to the loss of moisture and that the loss of moisture from cane of a given variety is a function of the saturation deficit of the air, independent of temperature, at least over a range of 45° to 65° F. (7.2° to 18.3° C.). In earlier work (*6*, *7*) it was shown that loss of moisture is an important factor in bringing about inversion of sucrose in sugarcane, and, conversely, the inhibition of moisture loss by sprinkling or by storage at high humidity checks inversion. These results are confirmed by data obtained in connection with two of the experiments reported in this paper. In one experiment the samples were stored at relative humidities of 96 and 72 percent at 65° F. and in the other at relative humidities of 98 and 71 percent at 66°. The high humidity materially checked inversion in both experiments. Because no other significant conclusions can be drawn from the data obtained from samples stored at high humidities, these data will not be presented.

The data obtained from cane stored at temperatures of 65° and 66° F. and relative humidities of 72 and 71 percent, respectively, and under dry conditions in the shade (tables 2, 3, and 4, and figs. 1 and 2) reveal some interesting relationships.

Because of the profound effect drying out of cane has on inversion of sucrose in some varieties (P. O. J. 36–M being one) and the fact that there is considerable loss of moisture through the cut ends of the stalks (*8*), it was expected that there would be more loss of moisture and hence greater inversion of sucrose in cane stored as sections. The total percentage of loss in weight of the three sections was greater than that of the whole-stalk samples in three of the four experiments (Nos. 3, 4, and 5, but not in No. 2, table 4), in which the time factor was comparable. In the experiment in which the exception occurred (No. 2), the samples were piled upon each other, thus preventing uniform exposure, whereas in the other three experiments the exposure of all samples was similar. It was concluded that the manner in which the samples were stored may have been responsible for this difference in weight loss in the different experiments. That this conclusion is justified is indicated by the greater variability in loss of moisture and inversion of sucrose in experiment 2 than in the other three experiments. The amount of inversion in an individual sample of experiment 2 was definitely associated with the amount of moisture loss.

The combined loss of sucrose from the three sections of cane sectioned either before or after storage was correlated in the main with the total loss of moisture (table 5).

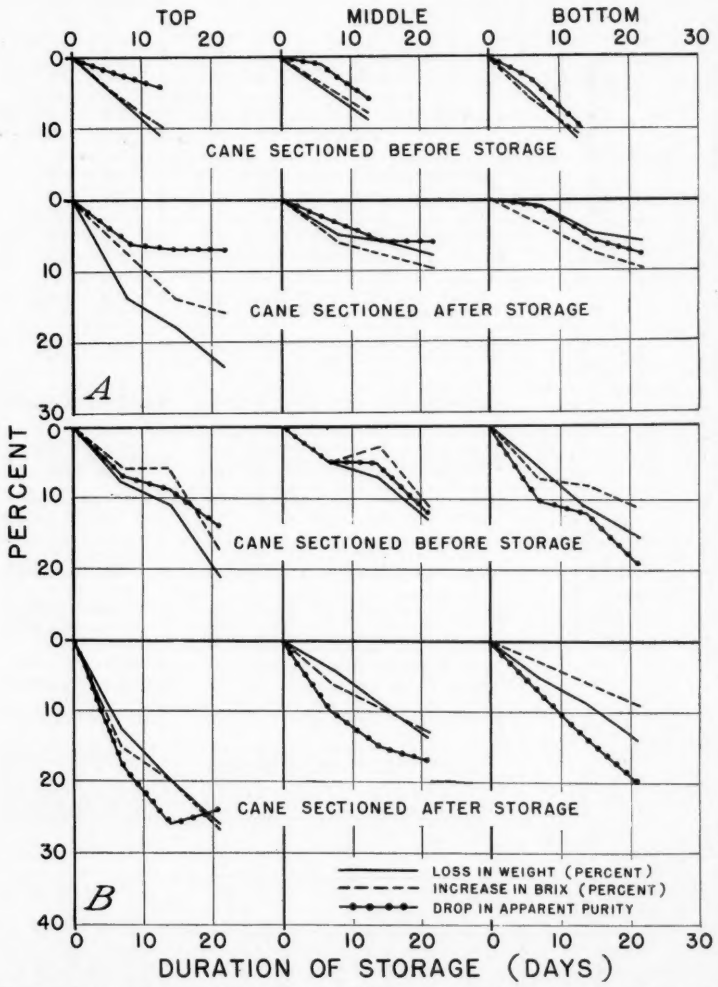

FIGURE 1.—Loss in weight, increase in Brix, and drop in apparent purity in the top-, middle-, and bottom-third portions of the sugarcane stalk sectioned before and after storage: *A*, P. O. J. 36–M, plant cane, stored from December 8 to 21 and December 30, 1932, at a temperature of 65° F. and a relative humidity of 72 percent; *B*, P. O. J. 36–M, first-year stubble, stored from November 8 to 29, 1933, at a temperature of 66° and a relative humidity of 71 percent. Analyses were made at different intervals during storage.

TABLE 2.—*Analysis of juice from top-, middle-, and bottom-third portions of the sugarcane stalk (P. O. J. 36–M) sectioned before storage and after storage under conditions of controlled temperature and relative humidity*

Time of sectioning cane	Section of cane	Conditions of storage: Temperature	Conditions of storage: Relative humidity	Date of analysis	Duration of storage	Brix	Apparent sucrose	Apparent purity	Drop in apparent purity	Yield of available sucrose per ton of cane [1]	Juice extraction	Acidity (0.1 N NaOH per 10 cc. of juice)	pH	Dry substance [1]	Sucrose [1 2]	Invert sugars [1 2]	Total sugars [1 2]	Ash [1 2]	Organic nonsugars [1 2]
		°F.	Pct.	1932	Days	°	Pct.			Lb.	Pct.	Cc.		Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
Plant cane:																			
Before storage	Top third	65	72	Dec. 8	0	14.14	10.13	71.6		129.6	64	2.40	5.18	13.31	77.08	13.24	90.32	4.29	5.39
				Dec. 14	6	14.85	10.37	69.8	1.8	123.9	60	2.45	5.30	13.46	76.18	13.96	90.14	4.17	5.69
				Dec. 21	13	15.57	10.51	67.5	4.1	114.8	60	2.50	5.31	13.37	75.20	14.46	89.66	3.99	6.35
	Middle third	65	72	Dec. 8	0	16.09	13.54	84.2		190.8	62	1.75	5.33	15.62	87.48	6.08	93.56	2.64	3.80
				Dec. 14	6	16.77	13.94	83.1	1.1	188.3	60	1.80	5.38	15.76	86.59	6.43	93.02	2.66	4.32
				Dec. 21	13	17.43	13.71	78.7	5.5	169.9	62	2.00	5.34	15.58	83.31	9.31	92.62	2.74	4.64
	Bottom third	65	72	Dec. 8	0	16.97	15.27	90.0		222.5	61	1.45	5.41	16.65	91.95	2.65	94.60	1.96	3.44
				Dec. 14	6	17.97	15.68	87.3	2.7	214.2	57	1.50	5.45	16.85	89.69	4.87	94.56	1.81	3.63
				Dec. 21	13	18.75	14.99	79.9	10.1	181.7	55	1.75	5.34	16.46	83.28	10.11	93.39	1.96	4.65
After storage	Top third	65	72	Dec. 8	0	14.14	10.13	71.6		129.6	64	2.40	5.18	13.31	77.08	13.24	90.32	4.29	5.39
				Dec. 16	8	15.27	10.10	66.1	5.5	104.3	56								
				Dec. 23	15	16.09	10.42	64.8	6.8	101.4	58	2.70	5.29	12.43	72.36	18.23	90.59	4.19	5.22
				Dec. 30	22	16.46	10.68	64.9	6.7	96.0	52	2.75	5.25	12.03	72.66	17.40	90.06	4.14	5.80
	Middle third	65	72	Dec. 8	0	16.09	13.54	84.2		190.8	62	1.75	5.33	15.62	87.48	6.08	93.56	2.64	3.80
				Dec. 16	8	16.99	13.76	81.0	3.2	180.6	59								
				Dec. 23	15	17.38	13.59	78.2	6.0	173.5	61	2.05	5.39	16.03	83.52	9.47	92.99	2.66	4.35
				Dec. 30	22	17.65	13.73	77.8	6.4	170.1	60	2.05	5.29	15.77	83.45	9.92	93.37	2.59	4.04
	Bottom third	65	72	Dec. 8	0	16.97	15.27	90.0		222.5	61	1.45	5.41	16.65	91.95	2.65	94.60	1.96	3.44
				Dec. 16	8	17.59	15.70	89.3	.7	225.5	59								
				Dec. 23	15	18.29	15.42	84.3	5.7	207.5	60	1.65	5.43	17.11	87.74	6.15	93.89	2.00	4.11
				Dec. 30	22	18.62	15.33	82.3	7.7	200.7	53	1.65	5.35	17.15	87.17	8.06	95.23	1.77	3.00
First-year stubble:																			
				1933															
Before storage	Top third	66	71	Nov. 8	0	12.62	7.85	62.2		90.1	55	1.94	5.14	11.85	69.28	19.76	89.04	5.36	5.60
				Nov. 15	7	13.43	7.38	55.0	7.2	69.3	55								
				Nov. 22	14	13.42	7.16	53.4	8.8	62.2	55								
				Nov. 29	21	14.78	7.20	48.7	13.5	49.5	52	2.01	5.22	11.00	58.08	29.68	87.76	5.53	6.71
	Middle third	66	71	Nov. 8	0	15.16	11.88	78.4		160.9	59	1.51	5.30	14.65	83.28	9.27	92.55	3.46	3.99
				Nov. 15	7	15.90	11.63	73.1	5.3	143.2	59								
				Nov. 22	14	15.67	11.48	73.3	5.1	138.8	58								
				Nov. 29	21	16.78	11.14	66.4	12.0	117.1	58	2.01	5.06	14.06	73.40	17.26	90.66	3.64	5.70
	Bottom third	66	71	Nov. 8	0	16.38	14.31	87.4		205.5	58	1.07	5.41	16.02	89.89	4.05	93.94	2.24	3.82
				Nov. 15	7	17.53	13.59	77.5	9.9	171.2	56								
				Nov. 22	14	17.75	13.42	75.6	11.8	159.1	57								
				Nov. 29	21	18.24	12.42	68.1	19.3	130.5	56	1.79	4.83	15.16	74.86	17.82	92.68	2.37	4.95

After storage	Top third	66	71	Nov. 8	0	12.62	7.85	62.2	.....	90.1	55	1.94	5.14	11.85	69.28	19.76	89.04	5.36	5.60
				Nov. 15	7	14.51	6.41	44.2	18.0	41.2	53	.....	.....	.....	.....	.....	.....	.....	.....
				Nov. 22	14	15.12	5.42	35.8	26.4	17.7	53	.....	.....	.....	.....	.....	.....	.....	.....
				Nov. 29	21	16.06	6.22	38.7	23.5	25.0	51	2.20	5.22	11.46	50.71	37.18	87.89	5.40	6.71
	Middle third	66	71	Nov. 8	0	15.16	11.88	78.4	.....	160.9	59	1.51	5.30	14.65	83.28	9.27	92.55	3.46	3.99
				Nov. 15	7	16.13	11.05	68.5	9.9	131.9	58	.....	.....	.....	.....	.....	.....	.....	.....
				Nov. 22	14	16.53	10.44	63.2	15.2	111.1	60	.....	.....	.....	.....	.....	.....	.....	.....
				Nov. 29	21	17.13	10.48	61.2	17.2	102.1	57	1.74	5.27	14.35	69.25	22.03	91.28	3.57	5.15
	Bottom third	66	71	Nov. 8	0	16.38	14.31	87.4	.....	205.5	58	1.07	5.41	16.02	89.89	4.05	93.94	2.24	3.82
				Nov. 15	7	16.84	13.48	80.0	7.4	176.1	56	.....	.....	.....	.....	.....	.....	.....	.....
				Nov. 22	14	17.36	12.67	73.0	14.4	148.8	57	.....	.....	.....	.....	.....	.....	.....	.....
				Nov. 29	21	17.78	12.00	67.5	19.9	127.2	57	1.45	5.15	15.03	74.84	18.37	93.21	2.25	4.54
Difference [2] for $P=0.05$									4.07										
Difference [2] for $P=0.01$									5.38										

[1] Corrected for weight loss.
[2] Percentage based on dry substance.
[3] The value given is based on cane stored at a temperature of 66° F. and a relative humidity of 71 percent.

TABLE 3.—*Inversion of sucrose as related to loss in moisture in the top-, middle-, and bottom-third portions of the sugarcane stalk sectioned before storage and after storage in the shade for different periods*

P. O. J. 36-M, SECOND-YEAR STUBBLE

Section of cane	Date of analysis	Duration of storage	Cane sectioned before storage						Cane sectioned after storage					
			Brix	Apparent sucrose	Apparent purity	Drop in apparent purity	Yield of available sucrose per ton of cane [1]	Juice extraction	Brix	Apparent sucrose	Apparent purity	Drop in apparent purity	Yield of available sucrose per ton of cane [1]	Juice extraction
	1934	*Days*	°	*Percent*			*Pounds*	*Percent*	°	*Percent*			*Pounds*	*Percent*
Top third	Nov. 2	0	13.31	8.77	65.9		105.5	58	13.31	8.77	65.9		105.5	58
	Nov. 9	7	14.39	5.60	38.9	27.0	27.3	59	15.47	5.40	34.9	31.0	17.3	58
	Nov. 16	14	15.20	5.01	33.0	32.9	10.6	60	16.62	4.40	26.5	39.4	[2]	58
Middle third	Nov. 2	0	15.87	12.89	81.2		178.2	59	15.87	12.89	81.2		178.2	59
	Nov. 9	7	16.75	9.51	56.8	24.4	93.1	61	16.93	8.97	53.0	28.2	81.9	61
	Nov. 16	14	17.72	8.49	47.9	33.3	63.0	64	17.38	7.04	40.5	40.7	38.7	66
Bottom third	Nov. 2	0	17.17	15.14	88.2		218.4	57	17.17	15.14	88.2		218.4	57
	Nov. 9	7	18.33	11.41	62.2	26.0	120.8	60	18.00	12.60	70.0	18.2	148.8	59
	Nov. 16	14	19.40	10.40	53.6	34.6	89.2	57	18.44	10.07	54.6	33.6	93.2	63
Difference for $P=0.05$						1.77						1.77		
Difference for $P=0.01$						2.36						2.36		
Top third	Nov. 28	0	13.70	8.96	65.4		107.2	61	13.70	8.96	65.4		107.2	61
	Dec. 5	7	14.79	8.52	57.6	7.8	81.9	56	15.19	8.31	54.7	10.7	71.8	57
Middle third	Nov. 28	0	16.40	13.69	83.5		192.1	64	16.40	13.69	83.5		192.1	64
	Dec. 5	7	16.79	13.77	82.0	1.5	182.7	61	17.11	14.00	81.8	1.7	189.1	63
Bottom third	Nov. 28	0	17.60	15.81	89.8		230.1	62	17.60	15.81	89.8		230.1	62
	Dec. 5	7	18.33	15.88	86.6	3.2	215.1	62	18.00	15.98	88.8	1.0	215.1	59
	CO. 290, FIRST-YEAR STUBBLE													
	1936													
Top third	Oct. 30	0	10.86	5.82	53.6		57.4	63	10.86	5.82	53.6		57.4	63
	Nov. 4	5	11.39	5.09	44.7	8.9	35.4	62	11.44	4.65	40.6	13.0	25.8	62
	Nov. 9	10	11.41	4.06	35.6	18.0	14.3	63	11.83	4.11	34.7	18.9	12.9	62
	Nov. 16	17	12.08	4.55	37.7	15.9	19.4	62	12.91	4.69	36.3	17.3	17.0	60
Middle third	Oct. 30	0	13.64	9.71	71.2		123.7	66	13.64	9.71	71.2		123.7	66
	Nov. 4	5	14.38	9.75	67.8	3.4	113.5	66	13.87	9.37	67.6	3.6	111.2	66
	Nov. 9	10	14.56	8.97	61.6	9.6	94.5	66	13.84	8.44	61.0	10.2	91.1	64
	Nov. 16	17	14.80	8.99	60.7	10.5	90.2	63	14.69	8.58	58.4	12.8	85.4	64

Bottom third	Oct. 30	0	15.44	12.46	80.7	----	171.6	63	15.44	12.46	80.7	----	171.6	63
	Nov. 4	5	16.27	12.69	78.0	2.7	162.8	63	15.63	12.33	78.9	1.8	174.0	65
	Nov. 9	10	16.28	12.08	74.2	6.5	147.3	65	15.54	11.62	74.8	5.9	148.2	63
	Nov. 16	17	16.94	11.81	69.7	11.0	133.0	63	15.95	11.61	72.8	7.9	144.5	64
Difference for $P=0.05$						1.86						1.86		
Difference for $P=0.01$						2.46						2.46		

[1] Corrected for weight loss.
[2] No yield because of low purity.

TABLE 4.—*Loss in weight, increase in Brix, loss of sucrose in juice, and loss of available sucrose in the top-, middle-, and bottom-third portions of the sugarcane stalk sectioned before and after storage*

P. O. J. 36-M

Experiment	Time of sectioning cane	Conditions of storage			Date of analysis	Duration of storage	Weight loss in—				Increase in Brix			Loss of sucrose in juice			Loss of available sucrose		
		Temperature	Relative humidity	Open shed			Top third	Middle third	Bottom third	Entire stalk	Top third	Middle third	Bottom third	Top third	Middle third	Bottom third	Top third	Middle third	Bottom third
		°F.	*Per cent*		*1932*	*Days*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
	Plant cane:																		
No. 1	Before storage	65	72		Dec. 14	6	4.9	3.5	4.8	4.4	5.0	4.2	5.9	2.7	0.7	2.2	4.4	1.3	3.7
		65	72		Dec. 21	13	10.9	8.7	11.5	10.4	10.1	8.3	10.5	7.6	7.5	13.1	11.4	11.0	18.3
	After storage	65	72		Dec. 16	8	14.4	4.9	1.0	6.0	8.0	5.6	3.7	14.6	3.3	+1.8	19.5	5.3	+1.3
		65	72		Dec. 23	15	18.0	5.6	4.6	8.9	13.8	8.0	7.8	15.7	5.2	3.7	21.8	9.1	6.7
		65	72		Dec. 30	22	24.4	8.1	6.0	12.0	16.4	9.7	9.7	20.3	6.8	5.6	25.9	10.8	9.8
	First-year stubble:				*1933*														
No. 2	Before storage	66	71		Nov. 15	7	7.5	5.1	6.4	6.3	6.4	4.9	7.0	13.0	7.1	11.1	23.1	11.0	16.7
		66	71		Nov. 22	14	11.4	6.9	10.5	9.5	6.3	3.4	8.4	19.2	10.0	16.1	31.0	13.7	22.6
		66	71		Nov. 29	21	21.0	13.2	14.9	16.1	17.1	10.7	11.4	27.5	18.6	26.1	45.1	27.2	36.5
	After storage	66	71		Nov. 15	7	13.0	3.7	4.7	6.9	15.0	6.4	2.8	28.9	10.4	10.2	54.3	18.0	14.3
		66	71		Nov. 22	14	20.2	8.5	9.3	12.1	19.8	9.0	6.0	44.8	19.6	19.7	80.4	31.0	27.6
		66	71		Nov. 29	21	25.6	13.8	13.5	17.3	27.3	13.0	8.5	41.0	24.0	27.5	72.2	36.5	38.1
	Second-year stubble:				*1934*														
No. 3	Before storage			Shade	Nov. 9	7	10.8	6.9	7.8	8.4	8.1	5.5	6.8	43.0	31.3	30.5	74.1	47.8	44.7
				do	Nov. 16	14	18.2	12.5	13.1	14.4	14.2	11.7	13.0	53.2	42.4	40.3	90.0	64.6	59.2
	After storage			do	Nov. 9	7	11.9	6.2	6.2	7.9	16.2	6.7	4.8	45.7	34.8	21.9	83.6	54.0	31.9
				do	Nov. 16	14	23.6	10.1	8.2	13.4	24.9	9.5	7.4	61.7	50.9	39.0	.....	78.3	57.3
No. 4	Before storage			do	Dec. 5	7	9.9	4.5	5.3	6.4	8.0	2.4	4.1	14.3	3.9	4.9	23.6	4.9	6.5
	After storage			do	do	7	14.4	2.7	1.7	5.8	10.9	4.3	2.3	20.6	.5	.6	33.0	.6	1.2
	CO. 290, FIRST-YEAR STUBBLE																		
					1936														
No. 5	Before storage			Shade	Nov. 4	5	7.7	5.3	5.0	5.9	4.9	5.4	5.4	19.2	4.9	3.2	38.3	8.2	5.1
				do	Nov. 9	10	11.0	7.4	6.9	8.3	5.1	6.7	5.4	38.0	14.4	9.7	75.1	23.6	14.2
				do	Nov. 16	17	13.7	10.7	10.3	11.4	11.2	8.5	9.7	32.5	17.3	15.0	66.2	27.1	22.5
	After storage			do	Nov. 4	5	9.7	3.2	1.5	4.6	5.3	1.7	1.2	27.8	6.6	2.5	55.1	10.1	+1.4
				do	Nov. 9	10	11.4	4.2	3.1	6.2	8.9	1.5	.6	37.5	16.7	9.6	77.5	26.4	13.6
				do	Nov. 16	17	16.2	8.0	3.7	8.9	18.9	7.7	3.3	32.5	18.7	10.3	70.4	31.0	15.8

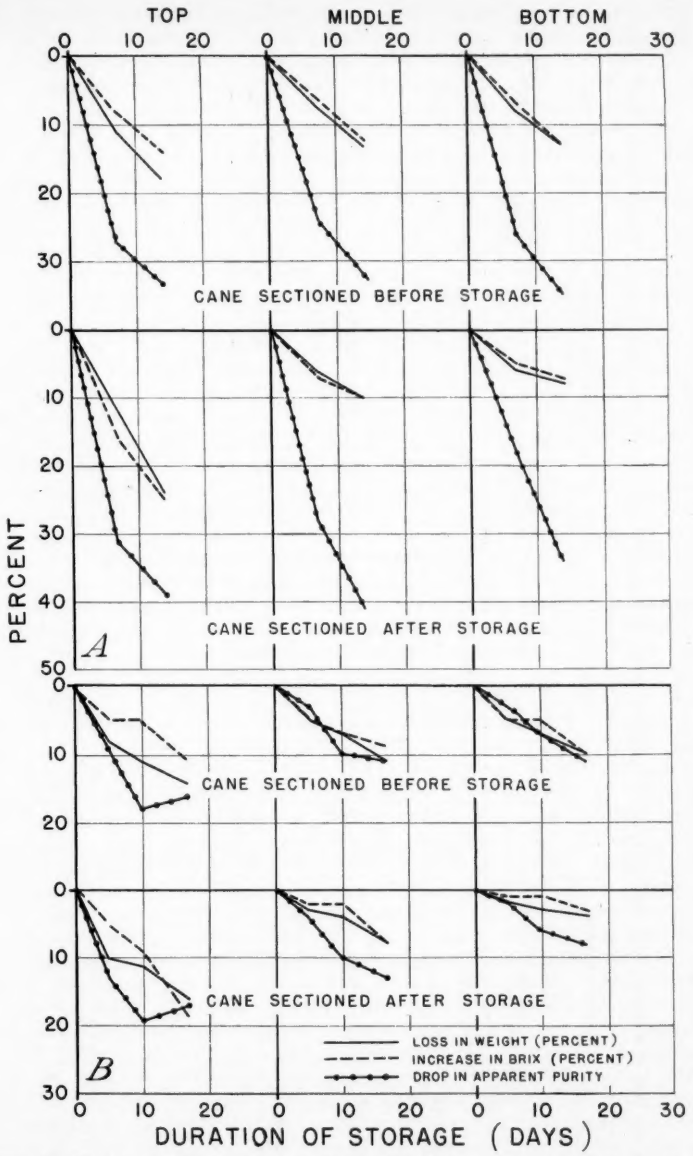

FIGURE 2.—Loss in weight, increase in Brix, and drop in apparent purity in the top-, middle-, and bottom-third portions of the sugarcane stalk sectioned before and after storage: *A*, P. O. J. 36–M, second-year stubble, stored from November 2 to 16, 1934; *B*, Co. 290, first-year stubble, stored from October 30 to November 16, 1936. The cane of both varieties and treatments was stored in the shade and analyzed after different periods of storage.

TABLE 5.—*Total loss of sucrose from sections of cane sectioned before storage, from those sectioned after storage, and from the whole stalk*

Variety	Conditions of storage			Date of analysis	Duration of storage	Cane sectioned before storage				Cane sectioned after storage				Whole stalk			
	Temperature	Relative humidity	Open shed			Yield of sucrose per ton of cane	Loss of sucrose		Loss in weight	Yield of sucrose per ton of cane	Loss of sucrose		Loss in weight	Yield of sucrose per ton of cane	Loss of sucrose		Loss in weight
	°F.	Percent		1933	Days	Pounds	Pounds	Percent	Percent	Pounds	Pounds	Percent	Percent	Pounds	Pounds	Percent	Percent
P. O. J. 36–M, first-year stubble.	66	71		Nov. 8	0	157.4				157.4							
				Nov. 15	7	132.5	24.9	15.8	6.3	122.5	34.9	22.2	6.9				
				Nov. 22	14	124.4	33.0	21.0	9.5	98.5	58.9	37.4	12.1				
				Nov. 29	21	102.7	54.7	34.8	16.1	89.4	68.0	43.2	17.3				
				1934													
P. O. J. 36–M, second-year stubble.			Shade	Nov. 2	0	172.0				172.0							
			do	Nov. 9	7	84.3	87.7	51.0	8.4	87.9	84.1	48.9	7.9				
			do	Nov. 16	14	57.5	114.5	66.6	14.4	44.4	127.6	74.2	13.4				
				1936													
Co. 290, first-year stubble.			Shade	Oct. 30	0	121.8				121.8				120.7			
			do	Nov. 4	5	108.6	13.2	10.8	5.9	109.2	12.6	10.3	4.6	114.0	6.7	5.6	4.0
			do	Nov. 9	10	90.3	31.5	25.9	8.3	89.1	22.7	18.6	6.2	97.8	22.9	19.0	5.8
			do	Nov. 16	17	85.1	36.7	30.1	11.4	87.0	34.8	28.6	8.9	88.2	32.5	26.9	8.8

Contrary to expectation, there was a greater drop in purity during storage (tables 2 and 3 and figs. 1 and 2) in the top third of the stalk of cane stored intact than in the top third stored as a section, and the reverse tended to be true of the bottom third. The relationship was very significantly true for P. O. J. 36–M, but it was less significantly so for Co. 290. The percentage of loss of sucrose in juice from P. O. J. 36–M (table 4) showed the same relationship as did the drop in purity. During the first period of storage there was a much greater loss of sucrose in Co. 290 in the top third of the stalk stored whole than in the top third stored as sections, but by the end of the second and third periods it was equal or slightly less. During most of the periods of storage there was a greater loss of sucrose in the bottom third of cane stored as sections than in the bottom third stored as whole stalks. Associated with these trends of inversion of sucrose in the top and bottom sections of the cane stalk, was a greater loss of moisture and increase in Brix in the top third of cane stored as whole stalks than in the top third stored as sections and in the bottom third of cane stored as sections than in the bottom third stored as whole stalks (table 4 and figs. 1 and 2). It would seem, therefore, that the loss of moisture in this instance, as in others, was a determining factor in bringing about inversion of sucrose. An explanation of the greater loss of moisture during storage in the top portion of an intact stalk of cane than in the top third of the stalk stored as sections and of the reverse tendency in the two treatments of the bottom third of the stalk is tentatively offered. The data indicate that there was a movement of water from the top to the bottom part of the stalk when intact and that this movement involved a greater loss of moisture from the top third than would have been the case if it had been severed from the remainder of the stalk. This movement of moisture may be accounted for by the difference in the osmotic pressure of the different parts of the cane stalk. Freezing-point data obtained in connection with varieties P. O. J. 36–M, Co. 281, and Co. 290 indicate that the osmotic pressure of the bottom and middle third of the stalk is always greater than that of the top third and is generally greater in the bottom than in the middle third.

In cane stored as whole stalks the percentage of loss of moisture and the increase in Brix tended to be greatest in the top third, next greatest in the middle third, and least in the bottom third. Such a trend might have been expected because of the physical difference in the three sections. The drop in purity (tables 2 and 3) and the percentage of loss of sucrose in juice and of available sucrose tended to follow the same trend (table 4 and figs. 1, 2, and 3), particularly during the early period of storage. The sectioning of the cane before storage disarranged the trend in moisture loss and increase in percentage of Brix; a similar disarrangement occurred or tended to occur with the drop in purity and the percentage of loss of sucrose in juice and of available sucrose. This disarrangement would seem to have resulted from the interruption of the movement of moisture from the top to the bottom part of the cane stalk, thus diminishing the loss of moisture in the top third and increasing it in the bottom third. In all five experiments this shift in loss of moisture resulted in a greater loss of moisture and consequently a greater increase in Brix in the top and bottom sections than in the middle section. There was a

corresponding shift in the drop in purity and percentage of loss of sucrose, and in three of the experiments these values were greater in the top and bottom sections than in the middle section. The increase in invert sugars in the different sections of both treatments in the two experiments in which they were determined (table 2) indicated the same trends as did the drop in purity and percentage of loss of sucrose. In most instances (table 2) a loss of total sugars

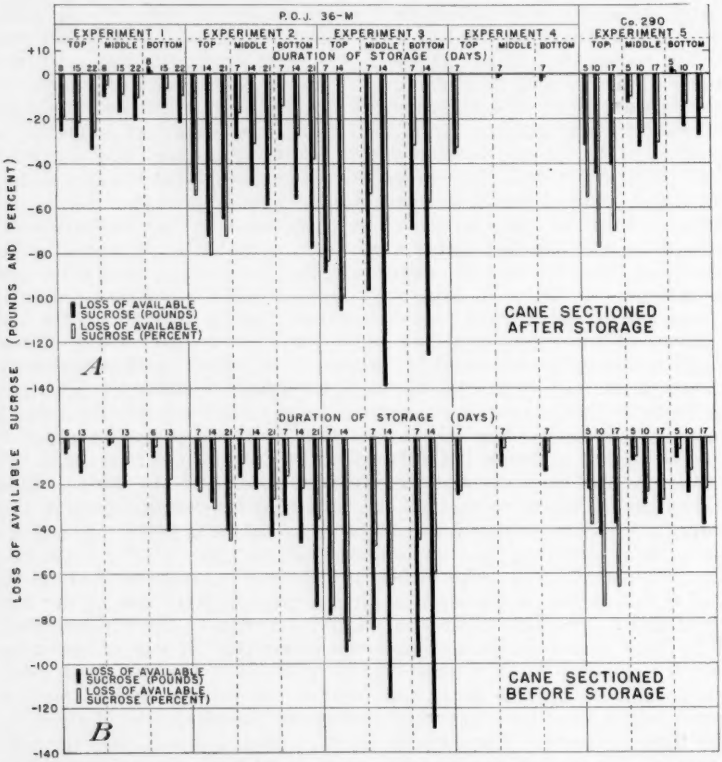

FIGURE 3.—Loss of available sucrose in pounds and in percentage as related to whole-stalk samples sectioned into one-third lengths after storage and then analyzed (*A*) and to samples sectioned before storage and analyzed after storage (*B*).

was indicated. This loss of sugars is regarded as having resulted from respiration.

There was a slight tendency for the percentage of increase in Brix as related to moisture loss to be accentuated in the top third of cane stored as whole stalks as compared with that in the top third of cane stored as sections. This tendency was reversed in the two treatments of the bottom sections. These observations suggest the possible movement of solids in cane stored as whole stalks and, in view of the greater drop in purity in the top third of this cane, of invert sugars

from the bottom to the top part of the stalks, or a greater consumption of solids by respiration in the top third of cane sectioned before storage. It is hardly conceivable that there would be a movement of invert sugars, for they would have to move against a gradient of concentration. It seems possible that, because of additional wounding in the top sectioned before storage, respiration may have been accelerated. It is also possible that some other conditions may be responsible for the apparent accumulation or disappearance of solids.

Thus far the rate of loss of sucrose as measured by the drop in purity and the percentage of loss of sucrose in juice and of available sucrose only have been considered. The actual loss of sucrose and the loss of available sucrose are matters of practical importance to the sugarcane planter. Although in a given case the rate of loss of sucrose may be greater in the top third of the sugarcane stalk than in the bottom third, the number of pounds lost may be greater in the bottom third than in the top third (fig. 3). This will be appreciated when it is realized that rate or percentage loss of sugar is dependent upon the initial concentration of sucrose present in the different sections.

SUMMARY

The data presented relate to inversion of sucrose and to changes in moisture content, and in Brix in the top-, middle-, and bottom-third lengths of the sugarcane stalk when sectioned into thirds and stored and when stored intact and sectioned after storage. In two experiments cane of both treatments was stored under controlled conditions of temperature and humidity, and in three experiments it was stored under dry conditions in the shade.

There was less inversion of sucrose in all samples stored at high relative humidities than in corresponding samples stored at low relative humidities.

In general, the rate of inversion of sucrose was correlated with the rate of loss of moisture.

In most instances there was more total loss of moisture and inversion of sucrose from the three sections in samples sectioned before storage than in samples sectioned after storage. The exception seemed to be due to the lack of uniform exposure of samples to the evaporating power of the air.

In cane stored as whole stalks there tended to be a gradient in the percentage of loss of moisture, percentage of increase in Brix, drop in purity, and percentage of loss of sucrose; these changes being greatest in the top, next greatest in the middle, and least in the bottom section.

In cane sectioned before storage this gradient was disarranged so that these changes tended to be greater in the top and bottom sections than in the middle section.

In the experiments in which the variety P. O. J. 36–M was used there was greater inversion in the unsevered top third of the stalks than in the severed top third. On the contrary, during the early periods of storage there was greater inversion in the severed bottom third than in the unsevered bottom third with the difference decreasing and sometimes disappearing with lapse of time. The behavior of the variety Co. 290 was similar to that of P. O. J. 36–M except that the differences were not so marked and after the first period of storage

there was a decrease in the difference in the top sections instead of in the bottom sections.

Correlated with these trends of inversion of sucrose in the top- and bottom-third sections of the sugarcane stalk was a greater loss of moisture and increase in Brix in the top third of cane stored as whole stalks than in the top third stored as sections and in the bottom third of cane stored as sections than in the bottom third stored as whole stalks.

Because of the difference in concentration of sucrose in the different parts of the sugarcane stalk, the actual loss of sucrose may not correspond to the rate of loss. It is only when the rate is much greater in the sections with the lower concentrations that these sections show the greatest loss of sucrose.

LITERATURE CITED

(1) Arceneaux, George.
1935. A simplified method of making theoretical sugar yield calculations in accordance with the Winter-Carp-Geerligs formula. Internatl. Sugar Jour. 37: 264–265.

(2) ———
1935. Studies of ripening of sugarcane in Louisiana and of effect of topping upon yields of cane and sugar per acre. U. S. Dept. Agr. Cir. 368, 32 pp., illus.

(3) Coates, Charles E.
1931. Keeping qualities of P. O. J. canes. (Letter to editor.) Sugar Bul. 9 (7, Jan. 1): 5–6.

(4) Cross, W. E., and Harris, W. G.
1916. Deterioración de las cañas de semillero de Java después de cortadas. Rev. Indus. y Agr. de Tucumán 7: [219]–250.

(5) Geerligs, H. C. Prinsen.
1904. Chemical control in use in the Java sugar factories 1904. 6: 380–388, 437–444, 483–491. [Reprinted in Methods of Chemical Control in Cane Sugar Factories. 1905.]

(6) Lauritzen, J. I., and Balch, R. T.
1934. Storage of mill cane. U. S. Dept. Agr. Tech. Bul. 449, 30 pp., illus.

(7) ——— Balch, R. T., and Fort, Charles A.
1938. Resistance to inversion of sucrose in harvested sugarcane in Louisiana. 6th Cong. Internatl. Soc. Sugar Cane Technol. Proc. Baton Rouge, La., Oct. 24–Nov. 5, 1938, pp. 809–818, illus.

(8) Sartoris, G. B.
1929. Rate of deterioration of sugar content of some P. O. J. sugarcane varieties in Louisiana. U. S. Dept. Agr. Cir. 97, 27 pp.

SALT BALANCE IN IRRIGATED AREAS [1]

By CARL S. SCOFIELD

Principal agriculturist, in charge, Division of Irrigation Agriculture, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

It is generally recognized that the use of saline irrigation water involves the danger that soluble salts may accumulate in the irrigated soil to its detriment. The artificial drainage of irrigated lands thus has two objectives: (1) The removal of surplus water, whether surface or subsoil accumulations, and (2) the removal of surplus dissolved salts that might otherwise accumulate in the soil solution of the root zone or in the subsoil water immediately below the root zone. The effectiveness of a drainage system is to be judged by the conditions of crop growth and by field observations made to ascertain, not only whether the surplus water is being removed, but also whether the drainage water is carrying away approximately as much dissolved salt as is being carried to the land by the irrigation water.

The objectives of the present paper are to describe methods and results (1) of field observations made to ascertain the quantities of dissolved salts carried to and removed from certain representative irrigated areas and (2) of a salt-balance experiment made at the Rubidoux Laboratory, Riverside, Calif.

In recording field observations, the relationship of salt input to salt output is designated as the salt balance for the area. If the mass of the salt input exceeds the mass of the salt output the salt balance is regarded as adverse, because this trend is in the direction of the accumulation of salt in the area and such a trend is manifestly undesirable.

To illustrate the use of these methods of determining the trend of salt-balance conditions, three irrigated areas have been selected, two of which are irrigated from the Rio Grande in the vicinity of El Paso, Tex., and the third from the Colorado River in the vicinity of Yuma, Ariz. These areas were selected for investigation because in each case the salinity of the irrigation water is relatively high, averaging approximately 1 ton of dissolved salts in an acre-foot of water, and because in each case it has been possible to measure the volume of input as irrigation water and the volume of output as drainage water and to obtain representative samples of each for analysis.

The data for the years of record for each irrigated area are presented in this paper. They show the trend of salt-balance conditions for each area not only in respect to the total dissolved solids but also

[1] Received for publication April 10, 1940. The data presented herewith concerning the salt balance of the Mesilla Valley and the El Paso Valley have been obtained through a cooperative investigation conducted by the Bureau of Reclamation, U. S. Department of the Interior, and the Division of Irrigation Agriculture, Bureau of Plant Industry, U. S. Department of Agriculture. The data as to the discharge of the stream at the gaging stations have been reported by the Bureau of Reclamation and the U. S. Section, International Boundary Commission, United States and Mexico; and the water samples, collected at least once a week at each gaging station, have been taken by the same agencies. The analyses of the water samples and the computation and compilation of the findings have been made by the Bureau of Plant Industry. This cooperative investigation has been carried on since February 1930, but because the sampling program during that year was not continuous, the period of the present report begins with January 1931.

in respect to each of the seven more important salt constituents. It is obvious, however, that these data as to salt input and salt output for the irrigated area as a whole may not give a true picture of the trend or rate of change of salt conditions in the root zone of the soil, and it is the conditions within the root zone that are critical in determining crop production. Information as to the total quantity of the dissolved salts or the quantity of each constituent of the input and of the output is not sufficient to show the trend of conditions in the root zone of the soil. Information is needed also as to the destination of the input salts: how much is absorbed by the crop plants and how much passes through the root zone into the subsoil water. Similarly, information is needed as to the origin of the output salts: how much is derived directly from the root-zone leaching and how much from the subsoil water that existed in the area prior to the period of record or even prior to the period of irrigation.

The scope of the present field investigation did not include any inquiry as to the volume of or the changes in the concentration of salts in the reservoir of subsoil water underlying any of the irrigated areas. The data reported must be taken as applying to each area as a whole and not alone to conditions in the surface or root-zone horizon. Nor does this field investigation afford information as to what proportion of the dissolved salts carried in the irrigation water may be absorbed by the crop plants. It has been assumed that this destination can account for only a small part of the total input when the salt concentration of the irrigation water ranges up to 1,000 parts per million, or more.

In order to supplement the field investigation and to obtain some information as to the movement and destination of the salts applied to a crop-producing soil with irrigation water, an experiment was set up and conducted for more than 4 years. The conditions of the experiment were such that the volume of irrigation input and of drainage output could be measured precisely; also, the concentration of dissolved salts in the input and output solutions was carefully determined and the crop growth (alfalfa) was harvested and weighed. From the data of this experiment, herein reported, it can be shown how much of the input water may be dissipated by surface evaporation and plant transpiration and how much must be allocated to root-zone leaching in order to prevent salt accumulation in the soil. Furthermore, it can be shown what quantity of water was dissipated by evaporation and transpiration for each unit quantity of crop produced, and finally how much of the input salt was recovered in the drainage or percolate when the soil was leached to its original salt content. The difference between the mass of salts in the irrigation input and in the drainage output indicates the quantity of salts absorbed by the crop plants.

It is not to be inferred that the data reported from this experiment may be applied quantitatively to field conditions. With the same or similar climatic conditions, it is to be expected that the use of land for one group of crops would give results differing quantitatively from results obtained from its use for another and different group of crops. Also it is to be expected that the same or similar groups of crops would show different quantitative results when grown under different climatic conditions. Notwithstanding these qualifications, it is

believed that these data, both from field observations and from a controlled experiment, may lead to a better understanding of the factors and relationships that exist in an irrigated area in respect to dissolved or soluble salts and to the problem of the adequate drainage of irrigated land.

FIELD INVESTIGATIONS

METHODS

Sampling and Analysis

The water samples involved in the present investigation were taken at gaging stations by the hydrographers responsible for measuring the stream discharges. At Leasburg Dam, on the Rio Grande, the hydrographer is employed by the Bureau of Reclamation, United States Department of the Interior. Samples were taken approximately once a week. Prior to November 1933 each sample was separately analyzed in detail. Beginning with November 1933, each weekly sample was analyzed only by determining its specific electrical conductance ($K \times 10^5$@25° C.) and then the four or five samples for each month were made into a composite for detailed analysis. In making up the monthly composites the volume of the aliquot taken from each sample was proportional to the volume of the discharge it represented.

At the Courchesne (El Paso) gaging station and also at the Fort Quitman gaging station, the hydrographers are employed by the United States Section of the International Boundary Commission, United States and Mexico. The sampling and analytical program for these two stations has been the same as that at Leasburg Dam except that since September 1937 the hydrographer at the Courchesne station has taken samples daily, determining the conductance of each sample, and has then made up the monthly composites, contributing from each sample a volume proportional to the discharge represented. Each of these monthly composites has then been analyzed in detail.

For the Yuma Valley, samples of the irrigation supply were taken at the Yuma gaging station each week by a hydrographer employed by the Bureau of Reclamation. Each sample was analyzed separately. Samples of the drainage water discharged from the Yuma Valley were taken weekly at the pumping plant located at the international boundary, at the south end of the valley. Prior to January 1935 each weekly sample was analyzed in detail. Subsequently, monthly composites were made up after the conductance of each individual sample had been determined, and these monthly composites were analyzed.

The methods of analysis used were those currently in use at the Rubidoux Laboratory, Riverside, Calif.[2]

Computation

In making the computations of the salt burden of a stream at a gaging station or of the salt-balance conditions of an irrigated area, the essential data include (1) the volume of water passing a given point in a given period of time and (2) the concentration and com-

[2] Methods and Analysis used in the Rubidoux Laboratory, Riverside, California. Ed. 2, rev 1938. [Mimeographed.]

position of the salts or salt constituents dissolved in the water. The rate of discharge of an irrigation stream is usually reported in terms of cubic feet per second of time (c. f. s.), which values may be converted into terms of acre-feet per day by multiplying them by the factor 1.98. The hydrographic reports of stream discharge are usually summarized to show discharge volumes as acre-feet per month. In the present computations the data of salt concentration and composition were also determined by months, either by the analysis of a monthly composite sample or by computing the mean of the analyses of the several individual samples taken during the month.

The data of the salt burden of an irrigation stream include (1) the quantity of total dissolved solids or of total salts and (2) the quantity of each of the salt constituents as determined by the detailed analysis. In this paper a distinction is made between the "total dissolved solids" and the "total salts." The value for total dissolved solids is computed from the results of a gravimetric determination in which a measured volume of a filtered aliquot of the water sample is evaporated and the residue is dried to constant weight at 105° C. The value for total salts is obtained by taking the sum of the gravimetric equivalents of the ions determined by the detailed analysis. These gravimetric equivalents are the products of the values for milligram equivalents per liter by the combining weight of each ion referenced to hydrogen as 1. In computing the gravimetric equivalent of the bicarbonate ion the combining weight is taken as 30.5.

In general the value obtained for total dissolved solids is somewhat greater than the value for total salts. The difference may be due in part to (1) some water of crystallization held by the salt residues, (2) some silica or other constituents not included in the detailed analysis, and (3) some dissolved organic matter that may pass the filter. In the waters of the Rio Grande and the Colorado, the concentrations of total salts are usually 90 to 92 percent of those of total dissolved solids. When the concentration values for total salts or total dissolved solids are reported as parts per million (equivalent to milligrams per liter), these may be converted into tons per acre-foot by multiplying by the factor 0.00136. This factor is derived from the assumption that an acre-foot of water weighs 2.72 million pounds.

The concentrations of the seven salt constituents are reported as milligram equivalents per liter. In order to convert these values into their equivalents as tons per acre-foot, a factor is used that is the product of the combining weight of the ion by the factor 0.00136. The factors used in these conversions are shown in table 1.

Thus, if volume of discharge for a month was 10,552 acre-feet and the calcium concentration was 4.94 mg. equiv. per liter, the computation for the tonnage of calcium in that volume of water would be:

$$4.94 \times 0.0272 \times 10{,}552 = 1{,}418 \text{ tons.}$$

The data for a gaging station were thus computed for each constituent for each month of a year and were tabulated and summarized. These annual summaries were then used in computing the weighted mean concentration and composition of the dissolved salts carried in the water.

TABLE 1.—*Factors for converting concentration values of salt constituents from milligram equivalents per liter to tons per acre-foot*

Constituent	Combining weight	Factor (=combining weight×0.00136)
Calcium	20	0.0272
Magnesium	12.15	.01652
Sodium	23.0	.03128
Carbonate and bicarbonate	30.5	.04148
Sulfate	48.0	.06528
Chloride	35.5	.04828
Nitrate	62.0	.08432

THE MESILLA VALLEY

DESCRIPTION

The area here designated as the Mesilla Valley includes that section of the flood plain of the Rio Grande between the Leasburg Dam near old Fort Selden, N. Mex., and the International Dam near El Paso, Tex. It is approximately 55 miles long and generally less than 5 miles wide. Its area is about 110,000 acres, of which approximately 80,000 are currently irrigated. Of the irrigated area, about 50,000 acres are devoted to cotton and 20,000 to forage crops, chiefly alfalfa. The land surface is about 3,800 feet above sea level. The mean annual temperature is 60° F. The mean annual precipitation is about 8.5 inches, most of which occurs during the late summer.

The irrigation supply for the valley is diverted from the Rio Grande, partly at Leasburg Dam, located in the NE¼ sec. 10, T. 21 S., R. 1 W., of the New Mexico principal meridian, and partly at Mesilla Dam, located in the NW¼ sec. 13, T. 24 S., R. 1 E. The water entering the valley by the river is measured at Leasburg Dam. There are no important tributaries to the river between Leasburg Dam and El Paso; consequently, the contribution of water other than that from the river comes from the precipitation falling on valley land and on slopes adjacent to the valley. The irrigated lands of the valley are served by a system of distributing canals fed from one or the other of the diversion dams on the river. They are served also by a system of open drains, aggregating 211 miles in length, that discharge by gravity into the river above El Paso. The water leaving the valley by the river is measured at the Courchesne gaging station, near International Dam and below the lowest outlet from the valley drains. Thus it is possible to measure and to sample the water both as it enters and as it leaves the valley by the river.

Not all of the water carried by the river into the valley is diverted into the canals. The river brings in annually about 750,000 acre-feet; the annual precipitation in the valley land constitutes about 75,000 acre-feet. The annual outflow from the valley passing the Courchesne gaging station is about 500,000 acre-feet, of which about 200,000 acre-feet is contributed from the valley drains. Thus it may be assumed that the annual diversions from the stream may amount to 450,000 acre-feet, i. e., to the difference between the annual inflow and the annual outflow, 250,000 acre-feet, plus 200,000 acre-feet returned to the stream through the drains. The annual dissipation

of water from the valley lands may be estimated at 325,000 acre-feet, i. e., the 250,000 acre-feet of stream depletion plus 75,000 acre-feet of precipitation.

Salt Balance

The essential facts in respect to the conditions of salt balance in an irrigated area involve at least two comparisons: (1) A comparison of the input with the output of the total dissolved salts and (2) a comparison of the input with the output of each of the more important constituents of these dissolved salts. It must be recognized also that these salts and salt constituents move into or away from an irrigated area only as they are dissolved in and carried by the water of irrigation and drainage. Consequently, a report on salt-balance conditions must include consideration of the volume of water moving into and the volume of water moving away from an irrigated area and the concentration of the dissolved salts in these waters. In table 2, the data presented fall into five categories: (1) The first three lines of the table relate to the annual volumes of water (a) moving into the area, (b) moving out from the area, and (c) dissipated from the area as evaporation and transpiration; (2) the next two lines give the concentration of dissolved salts in (a) the inflowing water and (b) the outflowing water; (3) the next two lines give the tonnage of total dissolved salts annually carried by the water (a) into the area and (b) away from the area; (4) the next line gives the ratio, as percentage, of the annual volume of outflow to the annual volume of inflow; and (5) the last line gives the cumulative tonnage of dissolved salts removed annually, for the period of record, from the area; i. e., the excess of salts removed as compared with salts brought in.

Table 2.—*Salt balance, Mesilla Valley, 1931–37*

Item	1931	1932	1933	1934	1935	1936	1937	Mean
Water:								
Input.....acre-feet..	738,000	814,000	824,000	768,230	632,400	693,260	740,770	744,380
Output.....do....	518,000	567,240	609,180	508,480	259,910	473,740	536,240	496,113
Dissipated.....do....	220,000	246,760	214,820	259,750	372,490	219,520	204,530	248,267
Salts:[1]								
Input.....tons..	621,655	697,204	588,367	642,955	566,809	550,625	527,970	599,369
Output.....do..	655,801	736,661	647,509	589,594	528,576	531,410	567,002	608,076
Concentration:								
Input..tons per acre-foot..	0.842	0.857	0.714	0.837	0.896	0.794	0.713	0.805
Output.....do..	1.266	1.299	1.063	1.159	2.034	1.122	1.057	1.226
Water output[2].....percent..	70.2	69.7	73.9	66.2	41.1	68.3	72.4	66.6
Cumulative salts removed tons..	34,146	73,603	132,745	79,384	41,151	21,936	60,968	8,707

[1] Sum of ions determined. [2] Percentage of input.

It will be observed from table 2 that for 7 years, 1931–37 inclusive, the mean annual inflow of water was 744,380 acre-feet. The mean annual outflow was 496,113 acre-feet, or 66.6 percent of the inflow. The mean annual difference, i.e., stream water dissipated, was 248,267 acre-feet. The mean concentration of the inflowing water was 0.805 ton per acre-foot, and that of the outflowing water 1.226 tons per acre-foot. These concentrations, expressed as parts per million, or milligrams per liter, are 592 and 901, respectively. The mean annual tonnage of dissolved salts carried into the area was 599,369, and the tonnage

carried out was 608,076, indicating an annual net removal of 8,707 tons.

In order to present a more detailed consideration of salt-balance conditions in the Mesilla Valley, the facts concerning the several constituents of the dissolved salts are given in table 3. The first two and the last lines of table 3 include the mean values from table 2 in respect to the input and output of stream water, of total dissolved salts, and of concentration of dissolved salts in the water. The remaining lines relate to the several constituents of the dissolved salts. For each of these the tonnage of input and of output is given, followed by the ratio of the output to the input expressed as a percentage. It will be noted that for the total salts the output was 101.5 percent of the input.

TABLE 3.—*Balance of salt constituents, Mesilla Valley; means for 1931–37*

Item	Input	Output	
	Acre-feet	*Acre-feet*	*Percent* [1]
Water	744,380	496,113	66.6
	Tons	*Tons*	
Salts [2]	599,369	608,076	101.5
Salt constituents:			
Calcium (Ca)	80,558	69,686	86.5
Magnesium (Mg)	17,557	15,468	88.1
Sodium (Na+K)	101,985	122,639	120.3
Bicarbonate (HCO_3+CO_3) [3]	93,936	80,540	85.7
Sulfate (SO_4)	233,419	207,950	89.1
Chloride (Cl)	71,306	111,122	155.8
Nitrate (NO_3) [4]	1,418	1,461	103.0
	Tons per acre-foot	*Tons per acre-foot*	
Concentration	0.805	1.226	152.3

[1] Percentage of input.
[2] Sum of ions determined.
[3] Computed as CO_3.
[4] For 1935 to 1937 only.

It is obvious at once that the output percentages of the salt constituents differ from that for the total salts. In other words, the composition of the salts in the outflowing water was different from that in the inflowing water. From this it follows that, while for the Mesilla Valley the annual output of total salts exceeded the annual input slightly, this was not the case with four of the seven salt constituents. With these four, viz, calcium, magnesium, bicarbonate, and sulfate, the output percentages ranged from 85.7 to 89.1. On the other hand, the output of sodium was 120.3 percent of the input; of chloride, 155.8 percent; and of nitrate, 103 percent.

The fact that the composition of the salts in the outflowing stream water differed from that of the inflowing water is not surprising. The subsoil of the valley land is saturated with water, some of which is very salty, and the composition of these salts differs from that of the salts of the river water. The salts of the subsoil water generally contain higher proportions of sodium and of chloride. These two constituents form a salt of high solubility, which may remain in a concentrated solution after the less soluble salts, such as the sulfates and carbonates of calcium and magnesium, have been precipitated. This subsoil water with its dissolved salts constitutes a large part of the drainage from the irrigated land, and this drainage makes up about

40 percent of the volume of the outflowing water. Thus it is to be expected that the composition of the salts of the outflowing water would differ from that of the salts carried by the inflowing water.

THE EL PASO VALLEY

Description

The area here designated as the El Paso Valley includes the irrigated flood plain on both sides of the Rio Grande between El Paso, Tex., and the Fort Quitman gaging station, which is located about 65 miles downstream from El Paso. This area includes not only the irrigated section that lies along the river in El Paso County, Tex., but also the section on the Mexican side of the international boundary, known locally as the Juarez Valley, and also the section in Hudspeth County, Tex. The total area on the American side of the international boundary is slightly less than 100,000 acres, of which about 60,000 acres are irrigated. There is probably a smaller area of the Mexican land, both total and irrigated, but data are not available as to this area. The allocation to crops on the American lands is similar to that of the Mesilla Valley. The elevation is only slightly lower, and the climatic conditions are much the same.

The irrigation water for lands on both sides of the river was formerly diverted at International Dam, Tex., located just west of El Paso. Recently, however, a new dam has been constructed about 2 miles upstream and just above the international boundary, and the American lands have been served from the new dam. There is one supplemental diversion for the American lands, known as the Riverside Heading, located on the river about 2 miles south of Ysleta, Tex., and another farther down at Fabens, Tex. The lands of the Hudspeth district are irrigated in part with water diverted for the lands in El Paso County, but not there used, and in part with water from the river diverted about 3 miles above Fort Hancock, Tex. The irrigated lands in El Paso County are served by 210 miles of drains, which discharge annually about 133,000 acre-feet of water, some of which is re-used for irrigation. The stream water entering the El Paso Valley is measured at the Courchesne gaging station, just above International Dam, and the total outflow is measured at the Fort Quitman gaging station.

Most of the river water that enters El Paso Valley is diverted and used for irrigation. While there are no important tributaries joining the main stream between El Paso and Fort Quitman, there are several arroyos that drain the higher lands bordering the valley, and after the more intense rains that occur occasionally in late summer these arroyos carry floodwaters for a short time. These floodwaters contribute very little to the irrigation supply and probably carry very little salt. They do, however, contribute to the volume of water passing the Fort Quitman gage and reduce its salt concentration for short periods. In view of uncertainties as to land areas and volume of run-off water from adjacent higher land, no estimate was made of the water contributed to the valley by precipitation nor of the total volume dissipated from the irrigated land.

Salt Balance

The data covering stream discharge and quality of water for the El Paso-Jaurez Valley have been obtained as a part of the cooperative investigation described above. The data for total salts are given in table 4. The data for the inflowing water for the El Paso Valley are the same as those for the outflow from the Mesilla Valley. For the 7-year period 1931–37, the mean volume of stream inflow was 496,113 acre-feet and the mean outflow 173,219 acre-feet, or 34.9 percent of the inflow. The difference between these values, 322,894 acre-feet, may be taken as the net stream depletion, or water dissipated in addition to the quantity of rainfall dissipated. It will be noted that the volume of output expressed as percentage of input ranged widely, from 20.1 in 1934 to 55.9 in 1935. Following the dry year of 1934, the water supply in Elephant Butte Reservoir was low, so that the volume available for the El Paso Valley in 1935 was below normal. There were also local torrential rains in the late summer of 1935 that contributed to the volume of run-off passing Fort Quitman. During the last 4 months of 1935, the discharge at Fort Quitman was 101,100 acre-feet as compared with 24,200 acre-feet for the corresponding period of 1934.

Table 4.—*Salt balance of El Paso Valley, 1931–37*

Item	1931	1932	1933	1934	1935	1936	1937	Mean
Water:								
Input.....acre-feet..	518,000	567,240	609,180	508,480	259,910	473,740	536,240	496,113
Output.....do....	212,000	211,120	213,790	102,360	145,380	149,590	178,290	173,219
Dissipated.....do....	306,000	356,120	395,390	406,120	114,530	324,150	357,950	322,894
Salts:[1]								
Input.....tons..	655,801	736,661	647,509	589,594	528,576	531,410	567,002	608,076
Output.....do....	637,234	597,996	542,285	285,963	289,919	402,520	476,642	461,794
Concentration:								
Input..tons per acre-foot..	1.266	1.299	1.063	1.159	2.034	1.122	1.057	1.226
Output.....do....	3.006	2.832	2.537	2.794	1.994	2.691	2.673	2.666
Water output[2].....percent..	40.9	37.2	35.1	20.1	55.9	31.6	33.2	34.9
Cumulative salt residue tons..	18,567	157,232	262,456	566,087	804,744	933,634	1,023,994	146,282

[1] Sum of ions determined. [2] Percentage of input.

The mean salt concentration of inflowing stream water was 1.226 tons per acre-foot, as compared with the mean concentration of the outflow of 2.666. These values are equivalent, respectively, to 901 and 1,960 p. p. m. The mean annual input of dissolved salts was 608,076 tons, whereas the mean annual output was 461,794 tons, indicating a mean annual residue of 146,282 tons, or a cumulative residue for the 7-year period of 1,023,994 tons. In respect to salt balance, conditions in the El Paso-Juarez Valley differed from those reported above for the Mesilla Valley. For the Mesilla Valley the output of dissolved salts exceeded the input by a mean of 8,707 tons a year, whereas for the El Paso Valley the input exceeded the output by 146,282 tons a year.

It is not to be inferred that the adverse salt balance of the El Paso-Juarez Valley, amounting to a million tons during the past 7 years, has been deposited uniformly in the irrigated soils of the valley. The evidence available does not afford a basis for estimating where this

salt has been deposited. The known facts are that for the valley as a whole the input of dissolved salts has exceeded the output by nearly 150,000 tons a year. It should be pointed out, however, that the quantity of soluble and dissolved salts in the soil of the valley and in the subsoil water contained in the valley sediments is very large. An acre-foot of soil may contain as much as 5 tons of soluble salts without seriously impairing its productivity, and in the El Paso Valley the salt content of the deeper sediments, below the root zone, may be much higher than that.

The facts concerning the balance of salt constituents for the El Paso Valley are given in table 5, which shows the mean annual tonnage of the input and output of each of seven salt constituents. For only one of these, chloride, was the output in excess of the input. For the total salts the mean annual output was 75.9 percent of the input. For the salt constituents the output percentages of both sodium and chloride exceeded that value.

TABLE 5.—*Balance of salt constituents, El Paso Valley; means for 1931–37*

Item	Input		Output		
	Acre-feet	*Ton equivalents* [3]	*Acre-feet*	*Ton equivalents* [3]	*Percent* [1]
Water	496,113		173,219		34.9
	Tons		*Tons*		
Salts [2]	608,076		461,794		75.9
Salt constituents:					
Calcium (Ca)	69,686	3,484	41,309	2,065	59.3
Magnesium (Mg)	15,468	1,268	10,217	837	66.1
Sodium (Na+K)	122,639	5,332	111,654	4,855	91.0
Bicarbonate (HCO_3+CO_3)	80,540	2,641	28,247	926	35.1
Sulfate (SO_4)	207,950	4,332	108,702	2,265	52.3
Chloride (Cl)	111,122	3,130	161,447	4,548	145.3
Nitrate (NO_3) [4]	1,461	24	508	8	34.8
	Tons per acre-foot		*Tons per acre-foot*		
Concentration	1.226		2.666		217.5

[1] Percentage of input.
[2] Sum of ions determined.
[3] Ton equivalent represents the actua tonnage divided by the combining weight of each constituent. The sums of the ton equivalents of the cations and of the anions should be approximately equal if the tonnage computations have been correct.
[4] For 1935–37 only.

THE YUMA VALLEY

DESCRIPTION

The Yuma Valley lies in the extreme southwestern corner of Arizona, in the flood plain of the Colorado River. It contains about 50,000 acres of irrigable land, of which about 40,000 acres are cropped. The ground surface is only about 100 feet above sea level. It is protected from river overflow by a levee. The climate is warm and dry. Crops may be grown throughout the year; some of the land yields two crops in a single year. The annual precipitation is only 2 to 5 inches and is almost negligible as a contribution to the water requirements of crops. The crops chiefly grown are cotton and alfalfa, with some barley, grain sorghum, and winter-grown vegetables.

The irrigation water is diverted from the Colorado River at Laguna Dam, a few miles upstream from Yuma. It is carried by canal on the west side of the river to a siphon near Yuma, through which it passes

under the river to the eastern or Arizona side at the upper or north end of the Yuma Valley. Some of the water delivered into the distribution system of the Valley Division is lifted by pumps to irrigate land on the Yuma mesa. Another portion is wasted back into the river or across the international boundary into Mexico. However, the total quantity of water annually delivered through the siphon is measured, as are also the quantities pumped to the mesa or wasted outside the valley from the distributing canals. Thus there is available, by difference, the net quantity of water annually delivered to the distribution system of the Valley Division. Data as to the quality of this water are based on samples taken each week from the river at the Yuma gaging station.

The irrigated lands of the Yuma Valley are served by a system of open drains that collect subsoil water, together with some waste water from the canals, and discharge this drainage water into a sump at the lower end of the valley from which it is pumped over the levee to make its way back to the river in Mexico. The quantity of the drainage water is measured, and data as to its quality are based on samples taken each week at the pump intake. The difference between the annual net input and the annual drainage output is taken as the quantity annually dissipated from the valley by evaporation and transpiration. For the year ending September 30, 1938, the net input was 259,917 acre-feet and the drainage output was 57,095 acre-feet, leaving a difference of 202,822 acre-feet as the quantity dissipated from 50,000 acres of valley land.

Salt Balance

The data on the salt-balance conditions of the Yuma Valley fall into two categories. One of these (table 6) relates to the 10-year period 1929–38, and the other (table 7) to the year 1938 only. The reason for the distinction lies in the fact that prior to 1938 the data for volume of irrigation input were based on the quantities of water "delivered to farms" rather than on the net quantities of water delivered to the distribution system of the valley. In 1938, for the first time, the report of input included both the net quantity delivered to the valley and the quantity delivered to farms. For the year ending September 30, 1938, the net delivery to the valley was 259,917 acre-feet, while for the same period the quantity reported as delivered to farms was 113,659 acre-feet. These two values would indicate that during the year the loss from the distribution system, presumably by percolation and evaporation, was 146,258 acre-feet, or 56 percent of the net input.

It seems obvious that the actual conveyance loss was not so great as is indicated by these values. The assumption is that the value for water delivered to farms is too low. In measuring farm deliveries the ditch riders tend to be liberal to the water user, and it is probable that the actual deliveries to farms exceed the reported deliveries.

The data for the 10-year period are given in table 6. Column 2 shows the quantities of water reported as annually delivered to farms. Column 3 shows the annual volume of drainage water pumped over the levee at the south end of the valley. The fourth column shows the differences between the values of columns 2 and 3. These difference values should not be taken as the quantities of water annually dissipated from the valley area of 50,000 acres, because the actual net

input of water has doubtless been much in excess of the quantity reported as delivered to farms. Columns 5 and 6 show the quantities of total dissolved solids in the water delivered to farms and in the drainage output, respectively. These values are computed from the residues of evaporated filtered samples and exceed by 9 or 10 percent the values for total salts obtained by taking the sum of the ions as determined by analysis.

TABLE 6.—*Annual volume of water "delivered to farms" in the Yuma Valley, annual volume of drainage output, and tonnage of total salts carried by each for the years ending Sept. 30, 1929–38*

Year	Delivered to farms	Drainage output	Difference	Total dissolved solids in—	
				Water "delivered to farms"	Drainage
	Acre-feet	*Acre-feet*	*Acre-feet*	*Tons*	*Tons*
1929	106,393	48,344	58,049	107,503	108,141
1930	100,453	49,158	51,295	98,685	98,398
1931	100,620	37,394	63,226	129,224	71,854
1932	95,208	33,477	61,731	104,139	64,258
1933	99,345	29,975	69,370	135,436	60,576
1934	108,352	27,169	81,183	166,498	59,695
1935	110,635	23,574	87,061	138,828	58,560
1936	119,597	36,830	82,767	108,797	82,986
1937	115,931	47,000	68,931	112,152	102,466
1938	113,659	57,095	56,564	117,624	121,728

TABLE 7.—*Salt balance, Yuma Valley, for the year ending Sept. 30, 1938*

[Irrigable acres 49,278; cropped, 36,250]

Item	Input	Output	
	Acre-feet	*Acre-feet*	*Percent* [1]
Water	259,917	57,095	22.0
	Tons	*Tons*	
Salts [2]	248,428	113,791	45.8
Salt constituents:			
Calcium (Ca)	35,552	11,517	32.4
Magnesium (Mg)	9,113	4,111	45.1
Sodium (Na)	34,895	23,579	67.6
Bicarbonate (HCO_3+CO_3)	26,303	11,698	44.5
Sulfate (SO_4)	113,771	30,256	26.6
Chloride (Cl)	28,024	32,542	116.1
Nitrate (NO_3)	770	88	11.4
	Tons per acre-foot	*Tons per acre-foot*	
Concentration	0.956	1.993	208.5

[1] Percentage of input. [2] Sum of ions determined.

For the 10-year period, if comparison is made between the tonnage of total dissolved solids carried by the water delivered to farms and that carried by the drainage output, it will be seen that the cumulative adverse salt balance has been about 390,000 tons or 39,000 tons a year. However, this finding is probably too low because the total input of dissolved solids doubtless exceeded the quantity reported. It is believed that the quantities of total dissolved solids reported for the drainage water approximate closely the actual output.

It remains now to consider the data on salt balance for the year ending September 30, 1938. This is the only 1 of the 10 years for

which the net volume of irrigation water entering the Yuma Valley has been reported. The data for the year are shown in table 7. The volume of input for the year was 259,917 acre-feet; the volume of output was 57,095 acre-feet, or 22 percent of the input. The tonnage of total salts contained in the input was 248,428; that of the output was 113,791, or 45.8 percent of the input. The adverse salt balance for the year; i. e., input minus output, was 134,637 tons. The weighted mean concentration of salts in the input water was 0.956 ton per acre-foot; that of the drainage water was 1.993 tons per acre-foot.

The same table shows the tonnage values for each of seven salt constituents in the input water and in the drainage, together with the ratios of the output values to the input values, expressed as percentages of input values. These values show that of the seven constituents only the output percentages for sodium and chloride exceeded the percentage for total salts. It was only in the case of the chloride constituent that output tonnage actually exceeded the input tonnage, about 4,500 tons more chloride being found in the drainage than in the irrigation water.

With regard to the data concerning the nitrate constituent, table 7 shows an input of 770 tons of nitrate (NO_3) and an output of only 88 tons. Unfortunately the analyses did not include determinations of potassium and phosphorus, which, together with nitrogen, constitute the three major plant-food elements. It is evident that although the conditions of irrigation and drainage in the Yuma Valley are such as to remove actually more chloride than is brought in, the same conditions do not take out as much nitrate as is brought in. Furthermore, it is to be assumed that some nitrate is added to the soils of the valley by applications of manure and commercial fertilizer and also by the production of an extensive acreage of alfalfa.

A SALT-BALANCE EXPERIMENT

PROCEDURE

An experiment designed to yield information as to salt-balance conditions in the root zone of the soil was set up at the Rubidoux Laboratory, Riverside, Calif., in June 1934. It was designated experiment No. 28.[3] The equipment consisted of two galvanized-iron cans 15 inches in diameter and 20 inches deep, each having a tubular drainage outlet at the bottom. A layer of coarse sand, about 1 inch deep, was placed in the bottom of each can to facilitate drainage. On this was placed about 18 inches of soil, having a surface area of 1.35 square feet. The mass of dry soil in each can was 86.5 kg., and its texture was such that after several wettings in excess it held 20 liters of water after percolation ceased. The original soil contained very little soluble salts, and some of this was leached away in the preliminary wettings. It was estimated that at the beginning of the experiment, on June 28, 1934, each can contained approximately 10 gm. of dissolved salts in its 20 liters of water.

Alfalfa (*Medicago sativa* L.) seed was planted in the soil of each can, and in due course the plants became well established and grew vigorously. Figure 1 shows the cans used in the experiment and the growth of alfalfa 3 years after the experiment was started. The regimen of

[3] The writer is indebted to George Y. Blair for faithful and painstaking cooperation in the conduct of this experiment.

irrigation has not been the same throughout the period of the experiment here reported, i. e., from June 28, 1934, to December 21, 1938. For certain periods the irrigation water used (the same water for both cans) contained dissolved salts in concentrations as high as 1.5 gm. per liter, or 1,500 p. p. m. For other periods the concentration of the input was much lower. The volumes of water used were sometimes not the same for both cans. For certain periods, the volume

FIGURE 1.—A crop of alfalfa grown in soil-filled cans in a salt-balance experiment at the Rubidoux Laboratory, Riverside, Calif.

applied to one can would be so large that the volume of percolate would be 25 percent of the volume of input while the volume applied to the other can would yield percolate of 10 percent or less of the volume of input.

Several times during the course of the experiment, irrigation water of low salinity was applied to both cans in large quantities in order to leach out the dissolved salts that had previously accumulated in the soil. Such a period of leaching preceded the conclusion of the period of record here reported so that from the concentration of the

percolate obtained just prior to December 21, 1938, it was estimated that each can contained approximately 22 gm. of dissolved salts in its 20 liters of water held in the soil.

RESULTS

Data for 1934–38

The data of the experiment make it possible to show (1) the volume of water and the mass of dissolved salts applied to each can during any period of time; (2) the volume of water and the mass of dissolved salts obtained as percolate from each can during any period of time; (3) for each period of time allocated to the growth of each successive crop of alfalfa, the volume of water dissipated from each can by evaporation and transpiration (volume of input water minus volume of percolate); this volume of water (expressed as liters), divided by the value for dry weight of the crop (expressed as grams), gives the water requirement for the crop; and (4) for each period of time between the successive thorough leachings, the mass of salts absorbed by the plants or by the soil or decomposed and dissipated (mass of dissolved salts contained in input water minus mass contained in percolate).

The record of the experiment as to water and salts for both cans for the whole period, from June 28, 1934, to December 21, 1938, is shown in table 8. During that period the total input of water was 5,490 liters, containing 5,443 gm. of dissolved salts. This indicates a mean concentration of 0.991 gm. per liter. For the same period the volume of the percolate was 1,237 liters, or 22.5 percent of the volume of the input. This volume of percolate contained 4,675 gm. of dissolved salts, or 85.9 percent of the input mass, and had a mean concentration of 3.779 gm. per liter. The volume of water dissipated as evaporation and transpiration, i. e., the volume of input minus the volume of percolate, was 4,253 liters. The mass of salt absorbed or dissipated, also the difference between input and output, was 768 gm., which includes 24 gm. of additional residue in the cans on December 21, 1938. This mass of salts, with reference to the volume of water dissipated, indicates a mean concentration of 0.175 gm. per liter.

Table 8.—*Record of experiment 28, from June 1934 to December 1938 and from Mar. 16 to Dec. 21, 1938, showing volumes, total salts, and concentrations of input, output, and residue*

Years and other items	Input	Percolate	Evaporation and transpiration
1934–38			
Solution volume, liters	5,490	1,237	4,253
Total salts, grams	5,443	4,675	[1] 768
Mean concentration, grams per liter	0.991	3.779	0.175
1938			
Solution volume, liters	1,438.3	389.4	1,048.9
Total salts, grams	1,609.1	1,397.1	[1] 212.0
Mean concentration, grams per liter	1.119	3.588	0.179

[1] Includes 24 gm. residue in the soil.

During the period of the experiment the alfalfa crop was harvested 29 times. The aggregate yield of dry matter for these crops from both cans was 5,510 gm. This mass of dry matter, with reference to the

volume of water dissipated by evaporation and transpiration, shows that 0.772 liter was dissipated for each gram of dry matter produced. In other words, it required the dissipation of 772 gm. of water to produce 1 gm. of alfalfa dry matter. Another comparison that may be made involves the mass of salts absorbed or dissipated and dry matter of crop produced. If it is assumed that 744 gm. of salts was absorbed by and removed with the alfalfa crops, the mass of these salts is equivalent to 13.5 percent of the alfalfa dry matter.

In this experiment the observations were made on what may be taken as a small field of alfalfa, having a root zone approximately 18 inches deep and a surface area of 2.7 square feet. The record of water applied includes both the volume of irrigation water, containing known quantities of dissolved salts, and the volume of rain water, assumed to be free of dissolved salts. At the beginning of the period of record the two cans contained approximately 20 gm. of dissolved salts in the 40 liters of water held in the soil; at the end of the period they contained 44 gm. of dissolved salts. Thus the regimen of irrigation during the period was such that practically all the salt applied was removed from the root zone. In other words, with the input water having a mean salt concentration of 991 p. p. m. approximate salt balance was achieved when the volume of input was such that 22.5 percent of it passed through the root zone as percolate.

With this regimen of irrigation it was found that, of 5,443 gm. of salt added to the soil with the irrigation input, 4,675 gm., or 85.9 percent, was carried through the root zone by the percolating water. It is not possible to show definitely the destination of 768 gm. of salt that was not removed with the percolate. It seems probable that part of it, possibly the major part, was taken up by the alfalfa plants; but some of it may have been precipitated in or absorbed by the soil, or some of the constituents may have been decomposed and dissipated in other forms.

Data for 1938

During the first 3 years of the experiment the record did not include detailed analyses of the percolates; only the total dissolved salts were determined. For the period beginning with March 16, 1938, the observations included detailed analyses both of the input solutions and of the percolates. These detailed analyses included the determination of four cations—calcium, magnesium, sodium, and potassium—and four anions—bicarbonate (computed as carbonate), sulfate, chloride, and nitrate. Before the findings in respect to these several salt constituents are presented, the data for the 1938 period in respect to total salts will be compared with similar data for the whole period of record (table 8).

For the 1938 period (281 days) the volume of input for the two cans was 1,438.3 liters, containing 1,609.1 gm. of dissolved salts. This indicates a mean concentration of 1.119 gm. per liter. The volume of percolate was 389.4 liters, equivalent to 27.1 percent of the volume of input. This percolate contained 1,397.1 gm. of salt, or 86.8 percent of the input mass, and had a mean concentration of 3.588 gm. per liter. The volume of water dissipated was 1,048.9 liters and the mass of salt added but not recovered in the percolate was 212.0 gm., which includes 24 gm. additional residue in the soil. This mass of salts (188 gm. net), with reference to the volume of water dissipated, in-

dicates a mean concentration of 0.179 gm. per liter. During this period six crops of alfalfa were harvested. They yielded 1,543 gm. of dry matter. This mass of dry matter, with reference to the volume of water dissipated, indicates a water requirement of 680 gm. of water for 1 gm. of dry matter. The mass of salt absorbed (188 gm.) is equivalent to 12.2 percent of the mass of alfalfa dry matter produced.

In comparing the data of the longer period with those of the included shorter period, it will be seen that the conditions and results were not greatly different. For the shorter period, the concentration of the input solution was slightly higher (1.119 : 0.991) and to achieve approximate salt balance for the root zone required a slightly higher ratio of percolate to input volume (27.1 : 22. 5). The proportion of the mass of salts recovered in the percolate was also slightly higher (86.8 : 85.9). The water requirement, on the other hand, was slightly lower (680 as compared with 772), but the proportion of salt residue to alfalfa dry matter was 12.2 percent for the shorter period and 13.5 percent for the whole period.

It is appropriate at this point to describe the character of the salts used with the irrigation water of this experiment. Throughout the period of record Rubidoux tap water was used. During the period prior to March 16, 1938, the salinity of the irrigation water was increased, as desired, by adding to the tap water suitable quantities of sodium chloride. After March 16, 1938, calcium chloride was used instead of sodium chloride. The cans were not protected from the weather; consequently they were exposed to the natural rainfall, the volume of which to be credited to each can was computed from the volume collected in a standard Weather Bureau rain gage located near the cans. From time to time, as the needs of the alfalfa plants seemed to warrant, small quantities of salts containing nitrate, potash, and phosphate were added to the irrigation water. Each lot of irrigation water was sampled and analyzed [4] so that the record of input included the nitrate and potash added but not the phosphate.

TABLE 9.—*Record of experiment 28 from Mar. 16 to Dec. 21, 1938, showing the grams of each of 8 salt constituents added with the irrigation water* [1] *and collected with the percolate,* [2] *and the difference between these values*

Salt constituent	Input	Percolate	Difference
	Grams	*Grams*	*Grams*
Calcium (Ca)	476.0	345.8	130.2
Magnesium (Mg)	19.1	29.9	[3] 10.8
Sodium (Na)	77.5	129.5	[3] 52.0
Potassium (K)	14.9	3.5	11.4
Carbonate (CO_3) [4]	126.9	78.1	48.8
Sulfate (SO_4)	83.0	68.6	14.4
Chloride (Cl)	797.3	752.6	44.7
Nitrate (NO_3)	31.6	.4	31.2
Total	1,626.3	1,408.4	217.9

[1] Includes dissolved constituents in soil solution on Mar. 16.
[2] Includes dissolved constituents in soil solution on Dec. 21.
[3] Gain in output over input.
[4] Computed as half the bicarbonate (HCO_3).

The record of the salt constituents added to and collected from the soil of the two cans between March 16 and December 21, 1938, is shown in table 9. It will be noted at once that in respect to mag-

[4] The writer is indebted to John T. Hatcher for making the analyses of the samples of irrigation water and percolate.

nesium and sodium the quantities found in the percolate exceeded the quantities added with the irrigation. For the six other constituents the quantities found in the percolate were less than those added with the irrigation. This recovery of more magnesium and sodium than was added is believed to be the result of reactions of base exchange that occurred in the soil. Prior to this period of the experiment the salts of the irrigation water had been chiefly sodium chloride, and it is assumed that sodium replaced some of the exchangeable calcium that the soil contained originally. The subsequent use of calcium chloride evidently caused the replacement of some of this sodium and of some magnesium as well.

It seems probable that the gains indicated for sodium and magnesium may not represent the full extent of the exchange reactions. It is to be assumed that these constituents may have been absorbed to some extent by the alfalfa plants. The quantities so absorbed should be added to the gains indicated to obtain the full values of the reactions of base exchange. In view of these gains of magnesium and sodium, it may be assumed that some part of the calcium unaccounted for (130.2 gm.) was involved in the reactions of base exchange. It is possible that the potassium may have been involved also, but in view of the low concentration of this constituent in the solution, it seems more probable that its chief destination was in absorption by the plants rather than in base-exchange reactions. With all of the anions, losses occurred. From the information available, it is not possible to estimate what proportion of these losses was due to plant absorption.

TABLE 10.—*Record of experiment 28 from Mar. 16 to Dec. 21, 1938, showing the gram equivalents of each of 8 salt constituents added with the irrigation water* [1] *and collected with the percolate,* [2] *and the difference between these values*

Salt constituent	Input (see table 9)	Percolate	Difference	Percolate
	Gram equivalents	*Gram equivalents*	*Gram equivalents*	*Percent* [3]
Cations:				
Calcium (Ca)	23.80	17.29	6.51	72.6
Magnesium (Mg)	1.57	2.46	[4] .89	156.7
Sodium (Na)	3.37	5.63	[4] 2.26	167.1
Potassium (K)	.38	.09	.29	23.7
Total	29.12	25.47	3.65	87.5
Anions:				
Bicarbonate (CO_3)	4.16	2.56	1.60	61.5
Sulfate (SO_4)	1.73	1.43	.30	82.7
Chloride (Cl)	22.46	21.20	1.26	94.4
Nitrate (NO_3)	.513	.006	.507	1.2
Total	28.863	25.206	3.667	87.3

[1] Includes dissolved constituents in soil solution on Mar. 16.
[2] Includes dissolved constituents in soil solution on Dec. 21.
[3] Percentage of input.
[4] Gain in output over input.

A clearer representation of the relationships of the salt constituents to one another can be obtained by expressing the values for input, output, and difference in gram equivalents rather than in grams. These relationships are shown in table 10. It will be observed that the values for input show that the sum of the cations exceeded slightly the sum of the anions. This may have been due in part to the

unavoidable discrepancies of analysis and in part to the omission from the computation of the small quantities of phosphate that were added with the irrigation water but not reported in the analysis. The values reported for the percolate show approximately the same discrepancy between the cations and anions. Here again there is uncertainty as to how much of the lower value for the sum of anions may have been due to errors of analysis and how much was due to greater losses or absorption of anions than of cations. It should be kept in mind that the values here reported represent the findings of 34 separate analyses.

The difference between the values for the input and for the percolate is shown both as gram equivalents and as percentage of the input value. The net loss for all cations (3.65) was 12.5 percent of the input; for the anions the corresponding percentage was 12.7. The largest percentage losses occurred with potassium and with nitrate. It is assumed that these losses were due largely to the absorption of these constituents by the alfalfa plants. In respect to the nitrate ion the values reported as milligram equivalents were as follows: Input 512.6, percolate 6.1, or a recovery of only 1.19 percent; but in reporting gram equivalents the nearest decimal values for two places were used. It is apparent that under the conditions of this experiment, which involved both a shallow root-zone and heavy leaching, there was not much loss by leaching of the two plant-food constituents.

The differences in the values for the cations show the most marked contrast. The percolate contained 3.15 gm. equiv. more of magnesium and sodium than was contained in the input solution. If it is assumed that these constituents were released from the soil by reactions of base exchange in which calcium was absorbed, then it appears that nearly half of the calcium that was lost was involved in these reactions. If allowance be made for the absorption of any magnesium and sodium by the alfalfa plants, then a corresponding quantity of additional calcium must be allocated to the exchange reactions. It may be repeated that during the period of the experiment prior to March 16, 1938, the salinity of the input solution had been due chiefly to sodium salt. This may explain why there was an abundance of replaceable sodium in the soil at the beginning of this last period of the experiment. However, it is not clear why so much magnesium was replaced from the soil.

For the four anions the mean loss, in gram equivalents, was 12.7 percent of the input values. The loss of nitrate was highest (98.8 percent). This is assumed to have been due largely to plant absorption. The next highest loss was in the carbonate constituent. Some of this loss may have been due to plant absorption, but it is possible that some of it was due to decomposition involving the absorption or dissipation of carbon dioxide and the precipitation of the carbonate as calcium carbonate in the soil. The percentage loss of sulfate was larger than the mean loss for all anions, but the actual quantity lost was small. On the other hand, the quantity of chloride lost was large but the percentage loss was small. It is assumed that most of the sulfate and chloride lost was absorbed by the alfalfa plants.

Each of the six alfalfa crops from each can for the 1938 period was analyzed for chloride and for total nitrogen. These analyses showed that the total crop (1,543 gm. of dry matter) contained 32.26 gm. of

chloride and 42.19 gm. of nitrogen. The data of table 9 show that the chloride content of the input (irrigation water) was 797.3 gm. and that the output (percolate) contained 752.6 gm. If the chloride found in the alfalfa be added to that contained in the percolate (752.6+32.26 gm.), the sum is 784.86 gm. This sum subtracted from the input chloride (797.3−784.86 gm.) leaves a difference of only 12.44 gm. of chloride, or 1.56 percent of the input chloride, unaccounted for during the period of the experiment.

The data as to nitrogen show a situation strikingly different from that of the chloride. For the 1938 period of the experiment the input of nitrate nitrogen (512.6 mg. equiv.) was 7.18 gm. The input solution may have contained some nitrogen in other forms, e. g., as ammonium salts, but the quantity was probably small. The percolate for the same period contained only 6.1 mg. equiv. of nitrate, equivalent to 0.085 gm. of nitrogen. On the other hand the crop contained 42.19 gm. of nitrogen. Thus the total output of nitrogen, in crop and percolate, was 42.275 gm. of nitrogen whereas the input was 7.18 gm. This indicates that the crop absorbed from the air and soil, through biological processes, approximately 35 gm. of nitrogen. This would be equivalent to 45 pounds of nitrogen for a ton of dry alfalfa.

DISCUSSION AND SUMMARY

FIELD INVESTIGATIONS

Results of the field investigations afford information on (1) the volume of stream water annually entering or delivered to a large irrigated area, together with the quantity and composition of the dissolved salts contained in the water, and (2) the volume of water annually leaving the same area as stream flow or drainage, together with the quantity and composition of the dissolved salts contained in that water. With optimum conditions it should be possible to ascertain from these data information on (1) the volume of water annually dissipated from the area as evaporation and transpiration, by taking the difference between the inflow and the outflow, and (2) the quantity and composition of the dissolved salts annually deposited in or removed from the soil of the area, by taking the differences in quantity and composition between the incoming and outgoing salts.

Under field conditions it is not practicable to determine accurately, by difference, the volume of water annually dissipated by evaporation and transpiration, because the actual input of water includes not only the stream flow but also rain water. The volume of annual precipitation falling within the boundaries of the irrigated areas might be estimated fairly accurately, but each of these areas is surrounded by higher ground from which there may be some run-off of storm water or some movement of ground water into the irrigated area. Therefore it is difficult to arrive at an acceptable value for total volume of input and consequently to compute the volume of dissipated water by subtracting the measured output.

In respect to the salt balance, the situation is different. The quantities of dissolved salt carried into and removed from the irrigated area by the stream waters and drainage may be computed with acceptable accuracy. And it may be assumed that the quantity of salt contributed directly or indirectly by the precipitation is so small as to be negligible. Thus, the quantity of salt brought in by the stream water

or irrigation supply may be taken as total input. The total salt output, however, includes not only the dissolved salts carried away from the area by the drainage and unused stream water, i. e., the total measured outflow, but also (1) the quantity of dissolved salts that may have been absorbed by and removed with the crops, (2) the quantity that may have been precipitated from solution in the soil, and (3) the quantity of such salt constituents as may have been decomposed in the soil, e. g., bicarbonate or nitrate, or may have been involved in reactions of base exchange, e. g., calcium, magnesium, and sodium. Therefore, the net salt balance, as computed by taking the difference between the quantities carried by stream or irrigation input and stream or drainage output, may be in error by the amount of the quantities absorbed, precipitated, or decomposed.

In the present consideration of these areas no attempt has been made to determine what proportion of the input salt passed into and remained in the subsoil water of the area, nor to estimate what proportion of the output salt originated from root-zone leaching as a direct result of irrigation and what proportion was contributed from subsoil water originally present in the area. The data presented relate to each area as a whole, including not only the root zone of the soil but also the deeper water-saturated sediments. It is conceivable that with a very large reservoir of subsoil water in an area and with conditions such that a large part of the input salt passes into that reservoir, an adverse salt balance may continue for a long time without involving the accumulation of harmful quantities of salts in the solution of the root zone.

On the other hand, conditions within the area may be such that a large proportion of the incoming irrigation water passes directly into the subsoil reservoir by way of percolation losses from canals. In that event the outflowing drainage water may represent largely water displaced from the subsoil reservoir and containing dissolved salts in high concentration and of remote origin. Under such conditions there may long continue for the area as a whole a favorable salt balance, i. e., output exceeding input, and yet with inadequate root-zone leaching there may be progressive and harmful accumulation of salts in the root zone.

From these considerations it follows that field observations as to salt-balance conditions cannot be expected to yield precise information as to the volume of water annually dissipated by evaporation and transpiration unless supplemented by precise information as to the rainfall contributions. It follows, also, that because of inherent uncertainty as to the volume, concentration, and composition of the original ground water in an irrigated area and as to the extent of its contribution to the drainage outflow, the findings of a short-period salt-balance investigation should not be interpreted as applicable to the surface soil of that area. These findings must be considered as applying to the whole system, which includes the surface or crop-producing horizon of the soil together with the underlying ground water. Field observations extending over a few years should show the trend of events in respect to salt balance and should indicate whether existing drainage facilities are adequate to maintain the present equilibrium for the area as a whole. It is manifestly desirable to know the facts in respect to the trend of change in salinity conditions.

The conditions of salinity that directly affect crop production are those that exist in the surface soil or root zone. It is chiefly the water supplied to the root zone by irrigation or rainfall that is used by plants. If this water contains dissolved salt constituents in excessive concentrations, crop growth is impaired. It is therefore appropriate to obtain information concerning salt-balance conditions in the root zone as affected by the character and the quantity of the irrigation water. Such information is not readily obtainable from field investigations. More precise information may be obtained by growing crop plants in soil under conditions that permit the accurate measurement of the volume of water applied and the volume of water that percolates through the root zone. It is necessary also to determine the concentration and composition of the dissolved salts in the irrigation water and in the drainage water.

LABORATORY EXPERIMENT

The objective of the laboratory experiment here reported was to obtain information as to salt-balance relationships to supplement the information currently obtained by observation of conditions in irrigated areas. Particularly it was desired to ascertain (1) what proportion of the irrigation water must be allocated to root-zone leaching in order to prevent the accumulation of soluble salts in the root zone and (2) what proportion of the dissolved salts of the irrigation supply is absorbed by the crop plants.

The results of the experiment show that for the whole period of 4½ years, during which the mean salt concentration of the irrigation water was 991 p. p. m. (1.35 tons per acre-foot), it was necessary to allocate 22.5 percent of the input to root-zone leaching in order to prevent salt accumulation in the soil. For the included shorter period of 9 months, during which the mean concentration of the irrigation water was 1,119 p. p. m. (1.52 tons per acre-foot), the necessary allocation to root-zone leaching was 27.1 percent of the irrigation input.

In respect to the second question, viz, the proportion of the dissolved salts that was absorbed by the crop plants (alfalfa), or by the soil, the findings for the longer period show that 13.7 percent of the total salts contained in the input water was so absorbed. During the shorter and final period the proportion so absorbed was 11.7 percent of the input.

The volume of water dissipated by evaporation and transpiration during the whole period of the experiment weighed 772 times as much as the dry weight of the alfalfa produced. For the included and final period of the experiment, the volume of water dissipated weighed 680 times as much as the dry weight of the alfalfa crops. The mass of dissolved salts absorbed by the crop plants and by the soil, i. e., the quantity in the input minus the quantity in percolate was, for the whole period, equivalent to 13.7 percent of the dry weight of the crop produced.

SUMMARY

The relation between the quantity of dissolved salts delivered to an irrigated area with the irrigation water and the quantity removed from the area by the drainage water is described as the salt balance of

the area. A favorable salt balance exists when the output of salts equals or exceeds the input. The salt balance is adverse when the input exceeds the output.

The conditions of irrigation and of drainage are described for three irrigated areas, two on the Rio Grande in New Mexico and Texas and one on the Colorado River in Arizona.

The methods of determining and computing the quantities of salts entering and leaving an irrigated area are described, and the salt-balance conditions for each area for a period of years are reported.

The data for these irrigated areas include also the relative input and output of each of the seven more important salt constituents and thus show the changes in the composition of the dissolved salts that occur in each area.

The field observations are supplemented by the data of a laboratory experiment that had two major objectives, namely, with a given concentration of dissolved salts in the irrigation water, to determine (1) what proportion of the irrigation input must be allocated to root-zone leaching in order to prevent the accumulation of soluble salts in the soil of the root zone and (2) what proportion of the dissolved salts contained in the irrigation water is removed from solution by the crop plant (alfalfa) or by the soil.

BORON ABSORPTION BY SUNFLOWER SEEDLINGS[1]

By CARL S. SCOFIELD, *principal agronomist, in charge*, L. V. WILCOX, *associate agronomist*, and GEORGE Y. BLAIR, *assistant pomologist, Division of Irrigation Agriculture, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

It has come to be generally accepted as a fact that many, if not all, crop plants require boron in order to make normal growth. In respect to a number of crop plants, definite and characteristic symptoms have been identified as due to a deficiency of available boron in the soil. The literature in this field has been abstracted and several comprehensive bibliographies prepared (*3*, *4*).[2] [3] It was shown by Mazé (*7*) and later confirmed by Warington (*11*) that boron must be included among the essential constituents of artificial solutions for the production of normal plants in water cultures. Boron is thus one of an indeterminate number of elements (*14*), such as iron, manganese, and zinc, that are essential to normal plant growth. Since the essential requirements of plants for these elements may be served by relatively small quantities as compared with the required quantities of such elements as carbon, nitrogen, potassium, and phosphorus, the term "miotrophic" [4] is proposed for this group. In respect to plant nutrition, the miotrophic group includes such elements as boron, iron, manganese, and zinc, which, though essential, are required in very small quantities.

Although boron is an essential constituent of the soil solution for normal plant growth, it is injurious to most plants when its concentration in the soil solution exceeds a few parts per million. Crop injuries have been reported as resulting from the use of commercial fertilizers containing salts of boron (*2*, *8*, *10*) and also from the use in irrigation of natural waters containing boron (*6*). In previous publications (*5*, *9*) it has been shown (*a*) that solution concentrations of boron above 3 or 4 p. p. m. may be injurious to many crop plants and (*b*) that the boron content of the plant material, particularly of the leaves, increases with the concentration of boron in the supporting solution.

In making field surveys to find and delimit the sources of boron that occur in certain irrigation waters of the Western States, it was found that the work could be expedited by collecting and analyzing the leaves of certain widely distributed plants. For example, the leaves of such trees as the walnut and any of the common species of citrus show, by characteristic markings and by analysis, whether supernormal concentrations of boron are present in the soil solution. Similarly, a number of herbaceous plants, including the sunflower and alfalfa, contain supernormal quantities of boron if the boron concentration of the soil solution is abnormally high. In view of these

[1] Received for publication May 1, 1940.

[2] AMERICAN POTASH INSTITUTE. BORON AS A PLANT NUTRIENT. A BIBLIOGRAPHY OF LITERATURE PUBLISHED AND REVIEWED JULY 1938 TO DECEMBER 1938, INCLUSIVE. Sup. 1, 40 pp. Washington, D. C., 1939. [Mimeographed.]

[3] Italic numbers in parentheses refer to Literature Cited, p. 56.

[4] A new word from Greek *mio-* (lesser) and *trophikos* (fr. *trophos*, feeder).

findings, it seems appropriate to explore the possibilities of the indicator-plant method in verifying and delimiting areas of boron deficiency.

In the course of investigating the subject of boron toxicity, a method of analysis (*1*, *12*) has been developed by which it is possible to measure, with a fair degree of precision, quantities of boron as small as 0.01 mg. in aqueous solution. By an adaptation (*3*) of this method it is possible to determine accurately the quantity of boron in plant material. It is possible also to determine, with acceptable accuracy, the total quantity of boron in a sample of soil, and the quantities of water-soluble and of acid-soluble boron in soils. There remains, however, some doubt as to a reliable method of determining, in a soil sample, the quantity of boron that is available to plants. This uncertainty has made it necessary to obtain information as to the relationship between the concentration of boron in a nutrient solution and the quantity of boron contained in plants grown therein.

SUNFLOWERS GROWN IN SOLUTION CULTURES

The sunflower was used in this investigation because (1) the plant grows well under a wide range of natural and artificial conditions, (2) it produces in a few weeks' time a relatively large quantity of plant material for analysis, and (3) it tolerates a wide range of boron concentrations. The first objective was to determine whether clear-cut symptoms of boron deficiency could be produced in sunflower seedlings by growing the plants in a nutrient solution made with ordinary laboratory reagents that were known to contain traces of boron.

EFFECT OF BORON ON GROWTH OF PLANTS

Experiment 1

Experiment 1 included three varieties of sunflowers and two culture solutions. The sunflower varieties were Mammoth Russian, California Double, and California Native. The first culture solution contained the following salts (millimoles per liter): Calcium nitrate ($Ca(NO_3)_2$), 1.25; potassium nitrate (KNO_3), 1.25; magnesium sulfate ($MgSO_4$), 0.50; sodium chloride (NaCl), 0.50; and monopotassium phosphate (KH_2PO_4), 0.25; together with very small quantities of the sulfates of iron, manganese, and zinc. The second culture solution differed from the first by having added to it a quantity of boric acid to give it a boron concentration of 2.5 p. p. m. Several analyses of the culture solutions to which no boron was added showed that it contained approximately 0.01 p. p. m. of boron.

The sunflower seedlings, shortly after germination, were set in the perforated tops of 1-liter glass jars, with three or four plants to each jar. During the 40 days after the seedlings were set into jars the solutions were restored to volume daily with distilled water, and four times during that period the used solutions were discarded and replaced with fresh solutions. The plants were set in the solutions in the greenhouse at the Rubidoux Laboratory, Riverside, Calif., January 30, 1939. By February 10 it became apparent that the plants grown in the solutions to which no boron had been added were not growing so well as the others. The difference in growth rate became greater as time went on. The plants grown without added boron made very little growth beyond the formation of the first leaves. The first inter-

node above the cotyledons was somewhat shortened, and the growing point soon died without producing the second internode.

The comparative growth made by plants of the three varieties of sunflowers in 6 weeks in the culture solution is shown in figure 1 and in table 1. It is obvious from figure 1 that the quantity of boron contained as impurities in the reagent salts used for the culture solution was insufficient to support normal growth of the seedlings. The

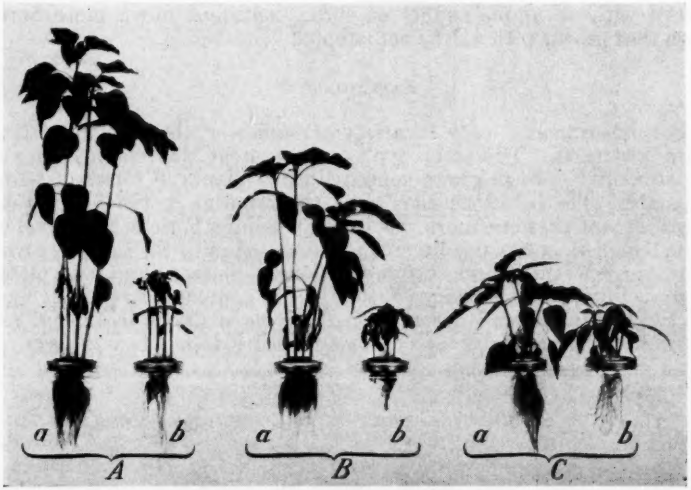

FIGURE 1.—Sunflower seedlings grown for 6 weeks in culture solutions with (*a*) and without (*b*) added boron: *A*, Mammoth Russian; *B*, California Double; *C*, California Native.

weights of dry matter reported in table 1 show that the plants without added boron made only 15 to 25 percent as much growth as those having an ample supply of boron.

TABLE 1.—*Dry weight, boron content, and boron concentration of each lot of sunflower seedlings of experiment 1*

Culture solution and variety	Plants	Leaves and stems		
		Dry material	Boron in dry material	
	Number	*Grams*	*Milligrams*	*Parts per million*
No boron added:				
Mammoth Russian	20	8.1	0.12	15
California Double	16	4.1	.05	12
California Native	6	2.1	.04	19
Boron 2.5 p. p. m.:				
Mammoth Russian	20	45.6	15.45	339
California Double	16	27.4	8.66	316
California Native	7	8.4	2.38	283

The data of table 1 show the number of plants in each group, the weight of dry material in the leaves and stems, the quantity of boron found in this dry material, and the ratio of the boron to the dry

weight. In respect to the concentration of boron in the plant tops, the differences resulting from the two solutions are very large. The mean concentration of the plants grown without boron was 15 p. p. m. while those having an ample supply of boron had a mean concentration of 313 p. p. m., or 20 times as much. The results of this experiment showed that sunflower seedlings require for normal growth more boron than occurs as impurities in the ordinary reagent salts used in making culture solutions. They showed also that the plant material grown with an ample supply of boron contained much more boron than that grown with a deficient supply.

Experiment 2

For experiment 2, only 1 variety of sunflower, the Mammoth Russian, was used. The same type of equipment was employed as in experiment 1. Each group consisted of 24 plants in 6 jars of 1-liter capacity. The solutions used were the same as in the first experiment, except that one more was used containing boric acid equivalent to 0.1 mg. of boron per liter of solution. Again, the seedlings were grown for 6 weeks in 5 successive solutions. The seedlings were placed in the solutions on February 24, 1939. The comparative growth made by the plants in the 3 boron concentrations is shown in table 2 and in figure 2 from a photograph made on April 4, 1939, after 39 days.

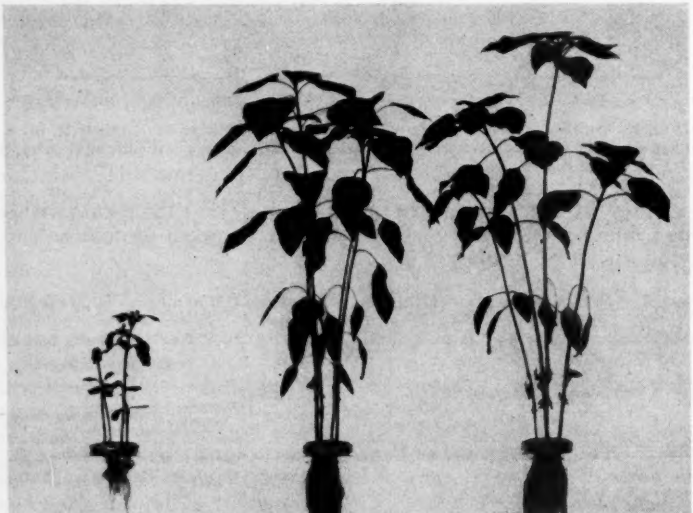

Figure 2.—Mammoth Russian sunflower seedlings grown for 6 weeks in culture solutions containing: *A*, No added boron; *B*, 0.1 p. p. m.; and *C*, 2.5 p. p. m. of added boron.

TABLE 2.—*Dry weight, boron content, and boron concentration of each lot of Mammoth Russian sunflower seedlings of experiments 2 to 4*

Experiment No.	Plants	Boron added to culture solution	Leaves and stems		
			Dry material	Boron in dry material	
	Number	*Parts per million*	*Grams*	*Milligrams*	*Parts per million*
2	24	0.0	7.6	0.11	14
	24	.1	56.5	2.18	39
	24	2.5	51.6	11.84	229
3	16	0	2.5	.03	12
	16	.025	24.5	.68	28
	16	.05	33.3	.99	30
	16	.075	35.0	1.23	35
	16	.1	36.3	1.36	37
4	16	0	1.5	.02	13
	16	.025	37.0	.89	24
	16	.05	45.4	1.37	30
	16	.075	46.0	1.85	40
	16	.1	43.4	2.07	48

The data of table 2 confirm the evidence obtained in the first experiment in respect to the comparable groups of plants. The growth made in solutions with no added boron was very restricted. On the other hand, the growth made in solutions containing 0.1 p. p. m. of boron was slightly larger than that made in the solution containing 2.5 p. p. m. The plants grown in the boron-deficient solution contained 0.11 mg. of boron, equivalent to 14 p. p. m. with reference to the dry material. The plants grown in the solution containing 0.1 p. p. m. of boron contained 2.18 mg. of that element. The 30 liters of solution to which these plants had access contained approximately 3.3 mg. of boron. This indicates that these plants absorbed about two-thirds of the boron available to them. The results of this experiment suggest that with sunflower seedlings the essential concentration of boron is less than 0.1 p. p. m. in the solution.

EXPERIMENT 3

The objective of experiment 3 was to explore the effects of boron concentrations of 0.1 p. p. m. and less. The same type of equipment was used as in experiments 1 and 2. Each group consisted of 16 plants in 4 jars. The same type of solution was used with boron added in concentrations of 0.025, 0.05, 0.075, and 0.1 p. p. m. There were 4 successive solutions used during the 6 weeks of the experiment, the volume being restored daily with distilled water. The sunflower seedlings, Mammoth Russian, were set in the solutions on April 8, 1939. The comparative growth made in each solution is shown in table 2 and in figure 3, *A–E*, from a photograph made on May 19, 1939.

Table 2 (experiment 3) and figure 3, *A–E*, show that even 0.025 mg. of boron per liter added to the culture solution made possible greatly increased growth in the sunflower seedlings as compared with growth in culture solutions to which no boron was added. The next increment of boron (0.05 p. p. m.) caused a further increase of growth of 36 percent. The increased growth in the next two solutions was not very great. The plants in the solutions without added

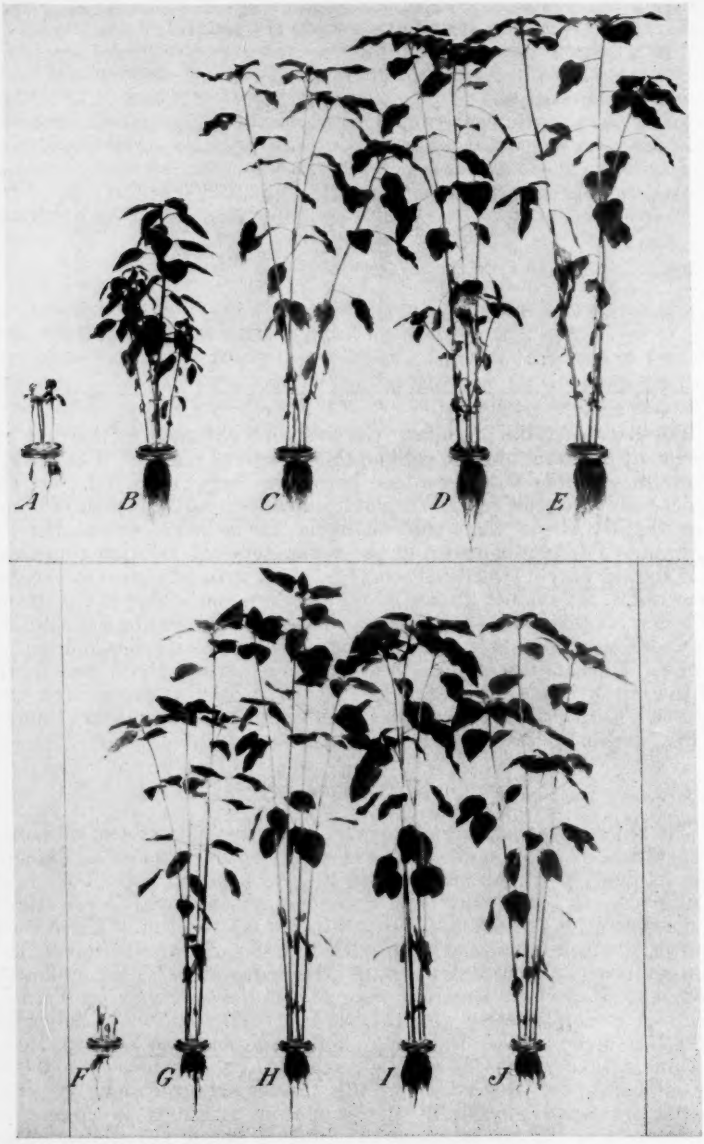

FIGURE 3.—Sunflower seedlings grown for 6 weeks in culture solutions containing: *A*, No added boron; *B*, 0.025 p. p. m.; *C*, 0.05 p. p. m.; *D*, 0.075 p. p. m.; and *E*, 0.1 p. p. m. of added boron; *F*, No added boron; *G*, 0.025 p. p. m.; *H*, 0.05 p. p. m.; *I*, 0.075 p. p. m.; and *J*, 0.1 p. p. m. of added boron.

boron made almost no growth beyond the cotyledon stage. Each group of plants had access to the boron contained in 16 liters of culture solution, 4 jars each, and 4 successive solutions. Thus the quantity in milligrams of boron available to each group of plants was, respectively, 0.16, 0.56, 0.96, 1.36, and 1.76, including the boron presumably contributed by the chemicals used in making up the solutions. It may be noted that the 16 sunflower seeds used for each group of plants contained approximately 0.025 mg. of boron.

The quantities of boron found in the plants of two of the groups exceeded slightly the quantities estimated as available in the solutions. Slight discrepancies in these relative values are not surprising because the quantities involved are very small and approach the limits of accuracy of the determinations. It is evident from the data reported that in this experiment the plants took from the solutions practically all the boron that was present. The concentrations of boron in the dry plant material increased consistently with the increases in the culture solutions, and concentrations in experiment 3 are very close to those shown for corresponding solutions in experiment 2 (table 2).

Experiment 4

Experiment 4 was essentially a repetition of experiment 3. It differed in that it was conducted later in the season, May 13 to July 25, when the temperatures were higher, and also in that the culture solutions were renewed more frequently. Otherwise, the method and the solutions used were the same as in experiment 3. With the advance of the season the seedlings grew faster and transpired more water. Observations as to the concentrations of the used solutions in experiment 3 had shown that the nutrient constituents were exhausted, and it was thought that more frequent solution renewals would give better results. In the 6 weeks' duration of experiment 4 there were nine successive solutions used as compared with four successive solutions in experiment 3.

The more frequent change of solutions increased not only the supply of nutrient constituents available to the plants but also the quantity of boron. The 9 successive solutions used made available to each group of 16 plants the following quantities of boron (in milligrams): 0.36, 1.26, 2.16, 3.06, and 3.96, including the boron presumably contained in the reagents used for the nutrient solutions. The comparative growth of the sunflower plants during the 6 weeks of the experiment is shown in figure 3, *F–J*, and in table 2.

The data of table 2 show that in experiment 4, as in experiment 3, there was a striking increase in growth as between the plants in the solution containing no added boron and those in the solution to which so small a quantity as 0.025 mg. per liter was added. In fact, most of the plants without added boron died before the experiment was concluded. The plants receiving 0.05 p. p. m. of boron made only 23 percent more growth than those receiving 0.025 p. p. m., and there was no increased growth with the solutions of higher boron concentration. It seems probable that the more frequent renewal of the solutions in this experiment supplied enough more boron to account in part for the growth differences shown in the two experiments.

The ratios of quantities of boron found in the plants to the quantities available in the solutions were as follows: 0.025 p. p. m., 71 percent;

0.05 p. p. m., 69 percent; 0. 075 p. p. m., 60 percent; and 0.1 p. p. m., 52 percent. However, the boron concentrations of the plant material increased progressively with increases of boron in the culture solutions.

WATER ABSORBED AND TRANSPIRED

In the course of experiments 2, 3, and 4, a record was made of the quantities of distilled water added daily to each jar of culture solution to restore the volume. These records give a measure of the quantities of water absorbed and transpired by each group of plants. Experience has shown that with a given plant and similar climatic conditions the quantity of water transpired is closely related to the quantity of plant material produced, and the term "water requirement" is used for the ratio between the two quantities. In table 3 are shown the volumes of water lost by each group of plants grown in experiments 2, 3, and 4. No record was made of the water used in experiment 1. The table also shows the weights of dry material produced by each group of plants and the water requirement for each group. It will be noted that the water requirement increased with the advance of the season from March to July.

TABLE 3.—*Volumes of water transpired by sunflower plants of experiments 2, 3, and 4, and water requirement of each group of plants*

Experiment No.	Plants	Boron in solution	Water transpired	Dry material of leaves and stems	Water requirement
	Number	*Parts per million*	*Liters*	*Grams*	*Liters÷ grams*
2	24	0.01	4.37	7.6	575
		.1	29.29	56.5	518
		2.5	26.08	51.6	505
3	16	.01	1.42	2.5	568
		.025	16.29	24.5	665
		.05	18.94	33.3	569
		.075	20.10	35.0	574
		.1	20.84	36.3	574
4	16	.01	1.21	1.5	807
		.025	27.54	37.0	744
		.05	33.81	45.4	745
		.075	36.81	46.0	800
		.1	33.71	43.4	777

The record of the volumes of water absorbed and transpired confirms other evidence, viz, that of the apperance of the plants and of the quantity of plant material produced, in showing that boron deficiency sharply curtailed plant growth. In general, the water requirement was slightly higher with the boron-deficient cultures but the differences were not great.

In these experiments the volume of the culture solutions was maintained by filling the jars from day to day with distilled water, and each culture solution, as restored to volume, was analyzed after it had been replaced by a new solution. These analyses show that the used solutions were of much lower concentration than the new solutions; in other words, that the plants had absorbed the dissolved constituents along with the water. Most of the water absorbed was subsequently transpired, but the dissolved constituents were retained by the plants. A record of the volume of water taken up by each group of plants and

a record of the boron found in each group make it possible to show, by the ratios between these values, the computed boron concentration of the water taken up by the plants. Table 4 shows the two values for each group of plants and the corresponding ratios.

TABLE 4.—*Volumes of water taken up by Mammoth Russian sunflower plants, boron content of the plants, and ratios of the two values*

Boron in solution (parts per million)	Experiment	Water taken up	Boron in leaves and stems	Ratio of boron to water taken up
	No.	*Liters*	*Milligrams*	*Parts per million*
0.01 [1]	2	4.37	0.11	0.025
	3	1.42	.03	.021
	4	1.21	.02	.016
0.025	3	16.29	.68	.042
	4	27.54	.89	.032
0.05	3	18.94	.99	.052
	4	33.81	1.37	.041
0.075	3	20.10	1.23	.061
	4	36.81	1.85	.050
0.1	2	29.29	2.18	.074
	3	20.84	1.36	.065
	4	33.71	2.07	.061
2.5	2	26.08	11.84	.454

[1] No boron added.

These data permit comparison between the determined boron concentration of the culture solution and the computed concentration of the water taken up. For the plant groups having culture-solution concentrations below 0.05 p. p. m. of boron, the boron concentration of the water taken up by the plants was higher than that of the culture solution. For the plant groups having solution concentrations above 0.05 p. p. m. of boron, the boron concentration of the water taken up by the plants was lower than that of the culture solution. In other words, when the supply of boron in the culture solution was such as to provide 0.05 mg. of boron for each liter of water absorbed by the plants, the normal processes of growth could go on. The inference that seems warranted is that with sunflower seedlings, symptoms of boron deficiency occur when the boron concentration of the available solution is less than 0.05 p. p. m.

PLANT WATER

The sunflower seedlings of these experiments contained a high proportion of water. When an experiment was concluded after approximately 6 weeks, the plants were removed from the culture jars, the stems were cut at the junction with the roots, and the leaves were detached from the stems. The green weights were then recorded for the roots, the stems, and the leaves of each group of plants except for the plants grown in the solutions without added boron. The plants of these cultures did not produce normal leaves, so the upper part of the plant with the cotyledons and partially formed leaves was classed as stems. After the green weights had been recorded, the plant material was dried to constant weight at 70° C. The loss in weight on drying is here designated as plant water.

270959—40——4

Because the water contained in the living plant is in contact with that of the supporting solution and because the proportion of water to dry matter may be very different in the roots, stems, and leaves, the authors feel warranted in reporting the relationship between the volume of plant water and the boron found in the plant material. In calling attention to this relationship, it is not implied that the boron is all held in solution in the plant water. Some of it, particularly in the leaves, is probably combined with the plant material and is not in solution. However, the plant water constitutes a system that is interconnected throughout the plant and may serve as a common denominator to show which parts of the plant contain relatively the most boron. Table 5 shows the plant water, the plant boron, and the ratios between these values for the roots, stems, and leaves of the sunflowers grown in the experiments here reported.

TABLE 5.—*Volume of plant water, boron content, and computed boron concentration of plant water in the roots, stems, and leaves of Mammoth Russian sunflower seedlings*

ROOTS

Boron in culture solution (parts per million)	Experiment	Plant water	Boron	Computed boron concentration of plant water	Mean
	No.	*Milliliters*	*Milligrams*	*Parts per million*	*Parts per million*
0.01[1]	1	33.2	0.14	4.2	2.2
	2	26.1	.06	2.3	
	3	10.1	.01	1.0	
	4	8.1	.01	1.2	
0.025	3	79.1	.27	3.4	2.9
	4	92.2	.22	2.4	
0.05	3	90.2	.30	3.3	3.0
	4	107.3	.29	2.7	
0.075	3	108.0	.30	2.8	2.3
	4	132.6	.25	1.9	
0.1	2	239.3	.47	2.0	2.5
	3	118.9	.37	3.1	
	4	123.8	.28	2.3	
2.5	1	253.1	.88	3.5	4.0
	2	198.3	.90	4.5	

STEMS

0.01[1]	1	64.9	[2]0.12	1.8	1.9
	2	71.0	[2].11	1.5	
	3	18.5	[2].03	1.6	
	4	7.6	[2].02	2.6	
0.025	3	94.3	.20	2.1	2.2
	4	199.8	.48	2.4	
0.05	3	148.1	.33	2.2	2.1
	4	287.1	.55	1.9	
0.075	3	149.3	.36	2.4	2.1
	4	290.0	.53	1.8	
0.1	2	278.3	.54	1.9	2.1
	3	165.8	.36	2.2	
	4	255.0	.54	2.1	
2.5	1	232.5	.82	3.5	4.1
	2	158.4	.74	4.7	

[1] No boron added.
[2] Stems with cotyledons and stunted leaves.

TABLE 5.—*Volume of plant water, boron content, and computed boron concentration of plant water in the roots, stems, and leaves of Mammoth Russian sunflower seedlings*—Continued

LEAVES

Boron in culture solution (parts per million)	Experiment	Plant water	Boron	Computed boron concentration of plant water	Mean
	No.	*Milliliters*	*Milligrams*	*Parts per million*	*Parts per million*
0.01 [1] [2]					
0.025	3	92.0	0.48	5.2	4.5
	4	107.1	.41	3.8	
0.05	3	89.3	.66	7.4	7.1
	4	121.1	.82	6.8	
0.075	3	92.1	.87	9.4	9.8
	4	129.9	1.32	10.2	
0.1	2	178.2	1.64	9.2	10.9
	3	86.9	1.00	11.5	
	4	128.3	1.53	11.9	
2.5	1	141.9	14.63	103.0	86.4
	2	159.0	11.10	69.8	

[1] No boron added.
[2] These plants produced no normal leaves.

The data of table 5 show that in the roots the ratio of boron to plant water is low, i. e., 2 to 4 p. p. m. and that the difference is not great as between the supporting solution containing 0.025 p. p. m. and the one containing 100 times as much. In the latter case the boron concentration of the plant water is only slightly higher than that of the culture solution.

It appears also that in the plant stems the ratio of boron to plant water is approximately the same as in the roots. This suggests that in the absorbing and transporting tissues of the plant normal growth processes may go on if the boron content of the plant water of these tissues is equivalent to 2 p. p. m.

In the leaves the boron ratios are higher. Furthermore, with the leaves these ratio values increase progressively with the increase of available boron in the culture solution. This may imply that in the photosynthetic tissues more of the boron is necessarily withdrawn from the plant-water system.

BORON CONTENT OF DRY PLANT MATERIAL

One of the objectives of the investigations here reported was to determine the suitability of the sunflower as a plant to be used as an indicator in verifying or delimiting areas of boron deficiency in the soil. The need in such survey work is a plant that grows rapidly and under a wide variety of conditions, either in natural soils or in soil samples, and particularly a plant that absorbs boron in proportion to the quantity of that element available in the soil solution. Because it is usually more convenient to collect and use for analysis the dry plant material, the appropriate data from these sunflower experiments have been assembled in table 6 to show the boron content of the roots, the stems, and the leaves.

These data show the boron content of the dry material expressed as parts per million. Consideration of the boron content of the root

material is less pertinent in this connection because it is not practicable to collect the roots of plants grown in the soil. The boron content of the stem material does not increase measurably with slight increases in the boron content of the culture solution. On the other hand, the boron content of the leaves increases progressively with the increase in boron in the solution. Through the range of solution concentrations from 0.025 to 0.075 p. p. m. of boron, the boron content of the leaves increases by 16 p. p. m. with an increase of 0.025 p. p. m. in the boron content of the solution. It is not to be expected that exactly this relationship would be found to occur generally. But it may be expected that if sunflower leaves contain less than 50 p. p. m. of boron this may be taken as an indication of boron deficiency.

TABLE 6.—*Boron content of dry material of roots, stems, and leaves of Mammoth Russian sunflowers grown in solutions having different proportions of available boron*

Boron in culture solution (parts per million)	Experiment	Boron in dry material of—				
		Roots	Stems	Mean	Leaves	Mean
	No.	*Parts per million*	*Parts per million*	*Parts per million*	*Parts per million*	*Parts per million*
0.01 [1]	1	76	[2] 15			
	2	48	[2] 14			
	3	12	[2] 12			
	4	22	[2] 14			
0.025	3	61	20	22	33	28
	4	34	24		24	
0.05	3	51	18	19	45	44
	4	40	21		43	
0.075	3	45	18	19	57	60
	4	30	21		64	
0.1	2	34	17	19	66	70
	3	54	17		66	
	4	36	23		77	
2.5	1	63	32	30	728	586
	2	77	28		444	

[1] No boron added. [2] Includes cotyledons and stunted leaves.

These findings in respect to the boron content of the plant material make it clear that it is not necessary or even desirable in boron-survey investigations to collect and analyze the whole sunflower plant. Even with seedling plants only 6 weeks old, the dry weight of the stems usually exceeds that of the leaves. Because of the fact that the stem material has relatively a low and constant boron content, the inclusion of stem material with the leaf material in samples taken for analysis would tend to diminish the differences that are sought.

SUNFLOWERS GROWN IN A SAND CULTURE

In addition to the solution-culture experiments here reported, another experiment (No. 5) was conducted. In this experiment the plants were grown in sand and watered with solutions similar to those used in experiment 1, viz, a solution to which no boron was added and a solution containing 2.5 p. p. m. of boron. The sand-culture equipment consisted of 12 Oldberg percolators, each containing approximately 2 kg. of sand and 4 sunflower plants of the Mammoth Russian variety. The solutions were applied to the sand in excess. The percolates were collected, restored to volume with distilled water, and re-used for several days. Five successive fresh solutions were used during the 6 weeks of the experiment.

The sand used in this experiment had been used previously in experiments dealing with boron toxicity, and consequently it had been in contact with solutions containing relatively high concentrations of boron. Before its use in this sunflower experiment the sand had been washed carefully, both with acid solutions and with distilled water, so that its boron content must have been very low. The sunflower plants grew very well in these sand cultures. Their appearance 6 weeks after germination is shown in figure 4.

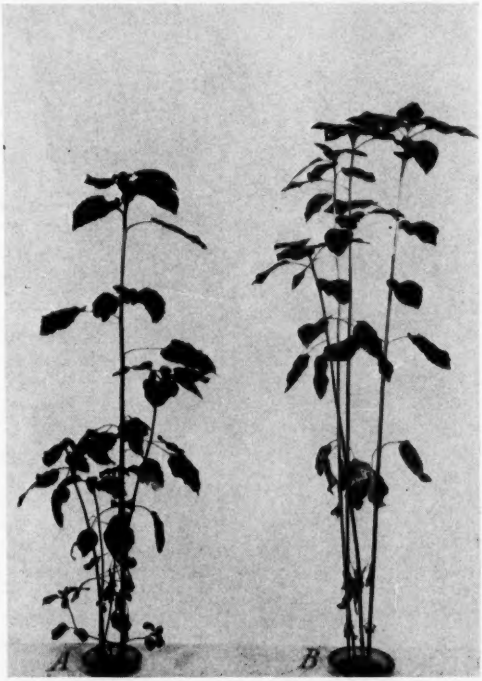

FIGURE 4.—Sunflower (Mammoth Russian) plants 6 weeks old, grown in sand cultures: *A*, No boron added to nutrient solution; *B*, 2.5 p. p. m. of boron in nutrient solution.

A comparison of the appearance of the plants grown in the sand culture without added boron and of plants grown in similar solutions without the sand (figs. 1, 2, and 3) makes it clear that the plants in this experiment obtained some boron from the sand. The quantity of boron so obtained was evidently small, because all the plants grown in sand without added boron were subnormal in some respects; most of the leaves were distorted in shape, and some of the upper internodes were much shortened. The characteristic condition of the growing point of a boron-deficient plant is shown in figure 5.

The findings of experiment 5, in respect to the growth, boron content, and water used, for the two groups of plants, are shown in table 7. The plants grown without added boron were smaller and contained much less boron than those having an ample supply of that element. They also used less water but had a higher water requirement. The plants of experiment 5 grown with a solution con-

taining 2.5 p. p. m. of boron contained 597 p. p. m. of boron in the leaves as compared with 586 p. p. m. for the leaves of plants grown in similar solutions in experiments 1 and 2.

FIGURE 5.—Growing point and upper leaves of a boron-deficient sunflower plant, 6 weeks after germination, in sand culture.

TABLE 7.—*Data on Mammoth Russian sunflower plants of experiment 5, grown for 6 weeks in sand cultures*

[24 plants in each group]

Culture solution and part of plant	Dry material	Boron in dry material		Water used by plants	Computed boron content of water used	Water requirement
Solution without added boron:	*Grams*	*Milligrams*	*Parts per million*	*Liters*	*Parts per million*	*Liters ÷ grams*
Stems	31.6	0.51	16	48.3	0.031	792
Leaves	29.4	.97	33			
Solution with 2.5 p. p. m. of boron:						
Stems	48.2	1.30	27	55.3	.352	704
Leaves	30.4	18.15	597			

The plants grown without added boron in the sand cultures of experiment 5 were similar in appearance to those grown in experiments 3 and 4 in solutions containing 0.025 p. p. m. of boron. In respect to the boron content of the dry leaves, the sand-culture plants contained 33 p. p. m. The plants grown in the low-boron solution (0.025 p. p. m.) of experiments 3 and 4 contained respectively 33 and 24 p. p. m. of boron in the leaves. The 24 plants of experiment 5 had access to 30 liters of culture solution estimated to contain 0.3 mg. of boron. This quantity subtracted from the 1.48 mg. found in the plants would leave approximately 1.2 mg. that must have been obtained from the 12 kg. of sand. In other words, it seems probable that the sand contained at least 0.1 p. p. m. of available boron. Attention is drawn to this low ratio of boron to the sand because it seems probable that had the sand contained much more available boron the plants would have absorbed it. Furthermore, it may be pointed out that it would be difficult, if not impossible, with known methods of direct analysis of soil, to measure quantitatively such a small proportion of boron. It is suggested that the use of plants to absorb the "available" boron from large masses of soil may be a useful method of testing soils believed to be deficient in available boron.

SUMMARY

It was desired to determine by experiment (1) whether sunflower plants grown in water-culture solutions without added boron would exhibit symptoms of boron deficiency; (2) what is approximately the concentration of boron in the culture solution above which normal sunflower plants are produced and below which symptoms of boron deficiency appear; and (3) whether the quantity of boron absorbed by sunflower seedlings is directly related to the quantities of boron available in the nutrient solution in the lower range of boron concentration.

The results of the experiments show that, if the culture solutions contain only the boron occurring as impurities in the ordinary c. p. salts used for nutrient solutions (approximately 0.01 p. p. m. of boron), sunflower seedlings do not grow normally and soon die.

It was found that normal sunflower plants may be produced with culture solutions containing approximately 0.05 mg. of boron per liter and that, if the boron concentration of the culture solution is much below that level, growth is restricted and the plants are abnormal in appearance.

With culture solutions containing approximately 0.05 p. p. m. of boron, the quantity of boron absorbed by the plant, as determined by analysis of the plant material, is closely related to the quantity available in the solution.

It is suggested that the quantity of available boron in a sample of soil or in soils in the field may be estimated by growing sunflower seedlings up to 6 weeks old and then analyzing the leaves for boron.

An experiment in which sunflowers were grown in sand cultures indicated that the sand contained approximately 0.1 p. p. m. of boron, a concentration so low as to be difficult to determine quantitatively by direct analysis of the sand.

It was found that throughout the lower range of concentrations of boron in the culture solution, the boron content of the roots and

stems of sunflower plants, with reference to the plant water, was equivalent to approximately 2 p. p. m. The concentration of boron in the leaves was higher and increased progressively with the increase of boron in the culture solution.

LITERATURE CITED

(1) American Public Health Association.
1936. standard methods for the examination of water and sewage. Ed. 8, 309 pp., illus. New York.
(2) Brown, B. E.
1922. effect of borax in fertilizer on the growth and yield of potatoes. U. S. Dept. Agr. Bul. 998, 8 pp., illus.
(3) Dennis, R. W. G.
1937. boron and plant life: recent developments in agriculture and horticulture. Fert., Feed. Stuffs and Farm Supplies Jour. 22: 479–483, 507–511, 535–538, 573–576.
(4) ——— and O'Brien, D. G.
1937. boron in agriculture. West of Scot. Agr. Col. Res. Bul. 5: 98 pp., illus.
(5) Eaton, Frank M.
1935. boron in soils and irrigation waters and its effect on plants with particular reference to the san joaquin valley of california. U. S. Dept. Agr. Tech. Bul. 448, 132 pp., illus.
(6) Kelley, W. P., and Brown, S. M.
1928. boron in the soils and irrigation waters of southern california and its relation to citrus and walnut culture. Hilgardia 3: [445]–458.
(7) Mazé, P.
1915. détermination des éléments minéraux rares nécessaires au développement du maïs. [Paris] Acad. des Sci. Compt. Rend. 160: 211–214.
(8) Neller, J. R., and Morse, W. J.
1921. effects upon the growth of potatoes, corn, and beans resulting from the addition of borax to the fertilizer used. Soil Sci. 12: 79–131, illus.
(9) Scofield, Carl S., and Wilcox, L. V.
1931. boron in irrigation waters. U. S. Dept. Agr. Tech. Bul. 264, 66 pp., illus.
(10) Skinner, J. J., Brown, B. E., and Reid, F. R.
1923. the effect of borax on the growth and yield of crops. U. S. Dept. Agr. Bul. 1126, 31 pp., illus.
(11) Warington, Katherine.
1923. the effect of boric acid and borax on the broad bean and certain other plants. Ann. Bot. [London] 37: 629–672, illus.
(12) Wilcox, L. V.
1932. electrometric titration of boric acid. Indus. and Engin. Chem., Analyt. Ed. 4: 38–39.
(13) ———
1940. determination of boron in plant material. an ignition-electrometric titration method. Indus. and Engin. Chem., Analyt. Ed. 12: 341–343, illus.
(14) Willis, L. G.
1939. bibliography of references to the literature on the minor elements and their relation to plant and animal nutrition. Ed. 3, 488 pp. Raleigh, N. C.

TWO NEW SPORE-FORMING BACTERIA CAUSING MILKY DISEASES OF JAPANESE BEETLE LARVAE[1]

By S. R. DUTKY

Agent, Division of Fruit Insect Investigations, Bureau of Entomology and Plan Quarantine, United States Department of Agriculture, and research assistan in soil microbiology, New Jersey Agricultural Experiment Station

INTRODUCTION

The existence of diseases among larvae of the Japanese beetle (*Popillia japonica* Newm.) and the part they play in the reduction of the populations of these larvae has been realized for some time. Probably the most important from the standpoint of the natural control of the insect are the so-called milky diseases. Two distinct milky diseases are recognized, referred to hereafter as type A and type B, whose causal agents are two closely related spore-forming bacteria. The author proposes the name *Bacillus popilliae*, n. sp., family Bacillaceae, for the species causing the type A disease and *Bacillus lentimorbus*, n. sp., for the causal agent of the type B disease. A search of the literature relating to insect diseases as well as that on spore-forming bacteria has failed to reveal any forms similar to these two bacilli.

Hawley and White[2] indicated that the diseases of the Japanese beetle could be classified, on the basis of the gross appearance of affected larvae, into three groups, the black group, the white group, and the fungus group. They considered that the majority of the dead larvae found in the field belonged to the black group. They concluded that there was probably only one disease present among larvae of the white group. This disease was characterized by the presence of large numbers of a microorganism in pure or nearly pure culture, which was probably the causal organism.

The writer[3] found that there were several diseases in the white group, of which types A and B milky diseases were the most prevalent and seemed to be responsible for the greater part of the reduction in larval population within the older area of beetle infestation. More recently Hadley[4] has given a brief summary of the status of the disease investigations. The present paper deals with the description of these milky disease organisms.

[1] Received for publication March 27, 1940. An investigation of the diseases of the immature stages of the Japanese beetle is being carried on jointly by the Bureau of Entomology and Plant Quarantine of the U. S. Department of Agriculture and the New Jersey Agricultural Experiment Station.

[2] HAWLEY, I. M., and WHITE, G. F. PRELIMINARY STUDIES ON THE DISEASES OF LARVAE OF THE JAPANESE BEETLE (POPILLIA JAPONICA NEWM.). N. Y. Ent. Soc. Jour. 43: 405–412. 1935.

[3] DUTKY, S. R. INVESTIGATION OF THE DISEASES OF THE IMMATURE STAGES OF THE JAPANESE BEETLE. Unpubl. thesis, Rutgers Univ., May 1937.

[4] HADLEY, C. H. PROGRESS OF JAPANESE BEETLE INVESTIGATIONS. N. Y. Ent. Soc. Jour. 46: 203–216. 1938.

Journal of Agricultural Research,
Washington, D. C.

Vol. 61, No. 1
July 1, 1940
Key No. K-307

THE TYPE A MILKY DISEASE ORGANISM

SYMPTOMS OF THE DISEASE

Gross Appearance

To the inexperienced eye there appears to be little difference between healthy larvae and those infected with the type A milky disease. However, even in the early stages the diseased grubs show a turbidity of the blood which obscures the dorsal blood vessel readily seen in healthy grubs. As the disease advances, the grubs acquire a milky-white appearance, which an experienced observer can easily distinguish from the fat accumulation in mature larvae (fig. 1).

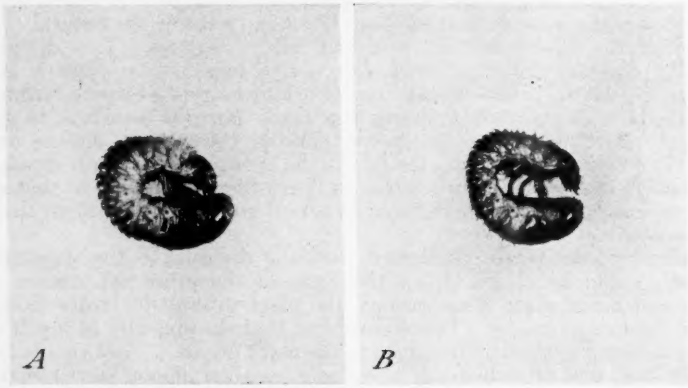

Figure 1.—Appearance of healthy larvae (*A*) and of larvae with type A milky disease (*B*). × about 2.

The activity of the larvae is not affected until within a few days of death, when they become sluggish. At the same time they turn slightly brownish, except in the lower parts of the body, which become chalky white owing to the settling out of spores as the blood circulation slows down.

Microscopic Appearance

When blood from a diseased larva is observed under the microscope, it is found to be swarming with two types of cells—a highly refractile, spindle-shaped, spore-bearing rod, and a slender, nonmotile rod. These cells are apparently developmental stages of the same organism. Few blood cells are observed, and these few appear little different from those of normal larvae. The milky-white appearance of the blood is due to these highly refractile spores, which may be present in numbers as high as 20 billion in the blood of a single individual (fig. 2). Examination of the fat tissue surrounding the intestine shows a large number of spores. Examination of other tissues by gross dissection does not reveal localization of spores in any number, although some are observed in the layers of cells of the midintestine.

DEMONSTRATION OF THE CAUSAL RELATIONSHIP

The causal relationship between the disease and the organism occurring in the blood was demonstrated as follows: When blood from

a diseased larva was injected into healthy larvae, the typical disease picture appeared. When saline suspensions of blood from a diseased larva were heated to 80° C. for 10 minutes and then injected into healthy larvae, the disease developed. The injected larvae showed the presence of the slender rod-shaped cells a few days after inoculation, and later both spores and rods, with the rods present in all transitional stages between the slender and the swollen spindle forms. These inoculation tests have been made with several thousand larvae

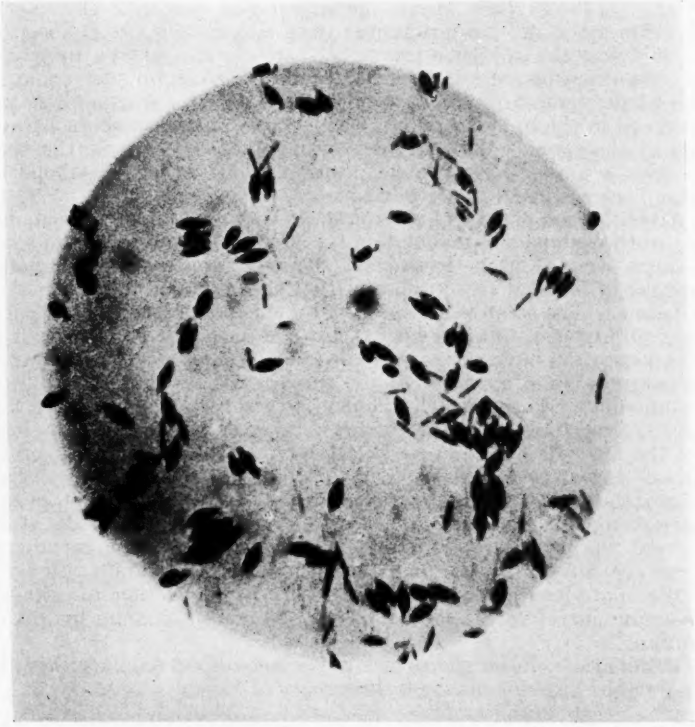

FIGURE 2.—Photomicrograph of the blood of a larva with type A milky disease, showing spore and rod forms of the disease organism. × 1,200.

and seem to establish the etiology of the disease and to show that the rod forms and spore-bearing spindle cells are only developmental stages of the same organism.

DESCRIPTION OF THE CAUSAL ORGANISM

MORPHOLOGY AND STAINING REACTIONS

The vegetative form of the organism is a slender, nonmotile rod occurring singly or in pairs. In the living condition the rods measure 0.9 by 5.2 microns. When fixed by Schaudinn's solution and stained by Hucker's crystal violet, the dimensions are about 0.3 by 3.5 microns.

The mode of division appears to be by plate formation rather than constriction, and is evidenced by the squareness of adjoining ends of the paired cells. After separation the ends are somewhat rounded. The cytoplasm in young cells is homogeneous and stains uniformly with Gram stain; in older cells granules are often found, and after fixing and staining, unstained areas are seen which divide the cell into two unequal sections.

The rods become swollen at sporulation. When the cell begins to swell, the spore becomes visible as a slightly refractile vacuole equal in size to the mature spore. As sporulation proceeds, the vacuole becomes more and more refractile until a definite spore is observed. At this time the cell has a pronounced spindle shape, and the spore is located somewhat terminally. One end of the cell broadens, and the cell becomes more pyriform than spindle-shaped. A granule is now observed in the broadened end, which grows until it is about half the size of the spore. With the development of the granule the spore assumes a more nearly central position. The cytoplasm about the spore becomes increasingly refringent.

After the completion of the refractile body and the increase in density of the cytoplasm surrounding the spore, no further morphological changes occur. In the fresh state the spore and granule are homogeneous in internal structure, and they do not take up either stains or iodine. The spore is surrounded by a halo formed by the encircling protoplasm, but it is very definite in outline. Spores free from the sporangium have never been observed. The size of the unstained sporangium is 1.6 by 5.5 microns, and that of the endospore 0.9 by 1.8 microns. When fixed by Schaudinn's solution and stained with Hucker's crystal violet, the refractile body and spore remain unstained, but the latter is obscured by the deeply stained surrounding protoplasmic layer. When fixed and stained, the spore-bearing cells are approximately 1.3 by 3.6 microns in size. When stained by Dorner spore stain, both the refractile body and the spore retain the stain, whereas the cytoplasm is completely decolorized. The membrane of the vegetative rods and both the membrane and the refractile body of the spore-bearing forms are resistant to the action of alkalies, remaining intact for at least 2 days in 10-percent sodium hydroxide solution.

Germination of the spores has never been observed in either the blood or the digestive fluids of the insect.

Artificial Culture of the Type A Organism

Repeated attempts to isolate the causal organism from the blood of infected larvae were unsuccessful. No evidence of growth, with either the vegetative or the spore stages, was obtained on nutrient agar by shake, slant, or Petri-dish culture methods. The inverted Petri-dish method of Krumwiede and Pratt,[5] which has frequently been used successfully for the isolation and cultivation of anaerobic bacteria, did not yield positive results. On blood-agar slants a slender, motile rod, forming small discrete colonies, was frequently obtained, but attempts to produce the disease by inoculation into healthy larvae were not successful. In peptone-glucose litmus whole milk

[5] Krumwiede, Charles, Jr., and Pratt, Josephine. Fusiform bacilli. Isolation and cultivation. Jour. Inf. Dis. 12: 199–201, illus. 1913.

heavy inoculations with spore forms yielded a slender, nonmotile rod identical in morphology with the vegetative forms seen in the blood of diseased larvae. No attempts were made to infect healthy larvae with the rods obtained in the milk cultures, since the number of rods produced in the cultures was not much greater than the number of ungerminated spores remaining in the medium.

Recently, when dried-blood films from diseased larvae have been used as the source of spores for inoculation, a large proportion of the attempts to isolate the causal organism have been successful.

Unheated egg-yolk media, used by White[6] for the isolation of *Bacillus larvae* White, proved to be satisfactory for the isolation of the type A milky disease organism. Heavy inoculations of spores from dried-blood films (700,000 spores per 5-ml. slant) were used, and the inoculated slants were incubated at 33° C. Slants of the basal medium, with and without the addition of sterile unheated egg yolk, were incubated under aerobic conditions at atmospheric pressure and at 700-mm. pressure, with the addition of 10 percent by volume of carbon dioxide. Inoculated slants were also incubated anaerobically under a pressure of 100 mm. of carbon dioxide. Under aerobic conditions growth of the type A organism occurred only on the egg-enriched medium, whereas under anaerobic conditions growth occurred equally with and without egg yolk. The beneficial action of the egg yolk must therefore be due in part to reduction of the oxygen content of the medium. The basal medium was fresh beef-infusion agar adjusted to pH 6.8, containing 0.5 percent each of dextrose and peptone. Sterile unheated egg-yolk suspension was added at the rate of 1 ml. per 5 ml. of the basal medium. Beef-infusion agar without peptone and without dextrose was also satisfactory as the basal medium.

The organisms form small discrete colonies on the slants, and as yet only nonmotile slender rods have been obtained in artificial culture. When the rods obtained in pure culture are inoculated into healthy larvae, the typical disease symptoms are produced and the blood of the inoculated larvae becomes loaded with typical spore forms of the type A organism.

Resistance of the Spores

The spores are heat-resistant, withstanding temperatures of 80° C. for 10 minutes, as shown by the production of the disease in larvae by inoculation of heated spore suspensions. The thermal death point of the spores has not been determined. The spores are also resistant to desiccation. Spores in blood films dried for periods as long as 42 months have given consistently high infection when moistened and inoculated into healthy larvae.

Development of the Causal Organism in the Insect's Blood

When healthy larvae are inoculated with spores of the causal organism and held at 30° C., certain developmental changes occur in the bacteria in the blood (fig. 3). For about 12 hours after inoculation no change is detected in either the morphology or the number of spores. Then there is a gradual decrease in the number of spores

[6] White, G. F. American foulbrood. U. S. Dept. Agr. Bul. 809, 46 pp., illus. 1920.

until, after 30 hours, about half the original number remain. At this time vegetative forms are also seen in small numbers; they usually occur in pairs (*C*, *D*). After 48 hours about one-third of the original spores remain, and rods are present in extremely large numbers, still largely in pairs. On the third day after inoculation the rods begin to swell and many cells show the presence of an oval central vacuole (*E*, *F*). During the next 24 hours there is an increase in refringency of the vacuole with the formation of the mature endospore (*G*, *H*). At this time most of the rods are observed in the early stages of sporulation; there is a slight bulging of the rod with the appearance of the vacuole, and a few cells with well-developed endospores, swollen

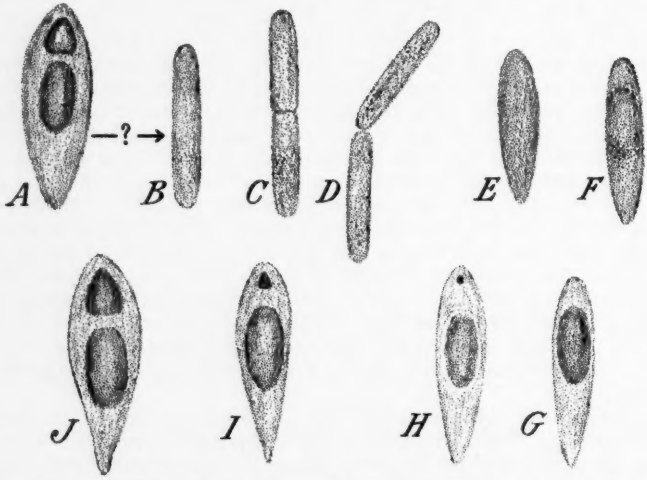

FIGURE 3.—Drawing illustrating the mode of reproduction and sporulation of the type A milky disease organism in the blood of the Japanese beetle larva: *A*, Parent spore; *B–D*, reproducing rods; *E–I*, sporulating rods in various stages of spore formation; *J*, mature spore-bearing rod. The question mark between *A* and *B* denotes that the exact mode of germination of the spores has not yet been determined. × about 6,000.

considerably and somewhat pyriform, showing a small granule in the broader pole (*I*). Periodic examination during the next 24 hours shows an increasing number of cells with initial granule formation and a few cells with the granule enlarged to the well-developed refractile body (*J*). The cells have the general appearance of those originally injected. By the sixth day the spores are sufficiently numerous so that the turbidity of the blood may be observed as a change in the external appearance of the larva. At this time many of the rods are still present, in the state both of division and of sporulation. The number of spores reaches a maximum about 7 to 10 days later, and at this time the larva is distinctly milky in appearance. The blood is found to be swarming with mature spore-bearing cells, and the rods, which are still present in considerable number, appear granular and many have lost their refringency. These so-called shadow cells are probably for the most part incapable of sporulation. The total num-

ber of spores in the blood at this time is from 2 to 20 billion, averaging about 5 billion, per larva. Subsequent examinations until the larva dies do not show any marked changes in the appearance of the spores, except that the surrounding protoplasmic layers are somewhat thicker and more refringent. Most of the rods appear to be either shadow cells, excessively granular cells, or sporulated cells that are not swollen appreciably. The rods stain only feebly. Examination of droppings from diseased larvae or the contents of the mid- or hind-intestine has not revealed the presence of spores or typical vegetative rods.

EFFECT OF TEMPERATURE ON TIME OF DEVELOPMENT OF DISEASE

To determine the effect of temperature on the time of development of macroscopic symptoms of the disease, larvae were injected with 2 million spores and held at various temperatures. The times of development were as follows: 4 days at 34° C., 6 days at 30°, 9 days at 25°, 11 days at 22°, and 14 days at 17°. Larvae held at 13° were not diseased after 63 days, and those held at 9° were still healthy after 28 days. When the larvae held at 9° for 28 days were placed at 30°, they developed the disease after 5 days.

The foregoing data show a linear relationship between the time of development of the disease and the temperature between 17° and 34° C. This corresponds approximately to the mathematical expression $T=24-0.6\ \theta$ (°C.), where T is the time of development of the disease and θ is the temperature of incubation.

Other tests were run with inoculated larvae held at 36°, 37°, and 40° C., for comparison with larvae similarly inoculated and held at 30° as checks. Although the checks showed consistent disease development, in no case did larvae held at the higher temperatures develop the disease. Temperatures as high as 36° to 40° are close to the maximum tolerated by larvae, and in no case did larvae survive after 1 week. In view of the rapid development of the disease at 34°, lack of development after 7 days at higher temperatures seems to indicate that 36° must be approximately the maximum temperature for development of the causal organism.

Larvae held at temperatures of 15° to 16° C. had not developed the disease after 29 days. The blood of larvae held at 15° for 29 days was found to contain a few spores but no vegetative forms. From these observations it is concluded that the most probable temperature range for development of this disease is from 16°–17° to 36°.

THE TYPE B MILKY DISEASE ORGANISM

SYMPTOMS OF THE DISEASE

Gross Appearance

Larvae infected with type B milky disease found in the late summer and fall cannot be distinguished macroscopically from those infected with the type A disease. In overwintering diseased larvae, however, the general appearance is quite different. Instead of having a milky-white coloration, these larvae are a muddy brown. Overwintering diseased larvae collected in March were milky white with little or no brownish discoloration, but when held at room temperature they

darkened rapidly until at the end of 2 or 3 weeks they had assumed the chocolate color generally found in type B-diseased larvae during April and May. Microscopic examination has shown this darkening of diseased larvae to be due to extensive formation of blood clots which are brown to jet black. Chocolate-brown larvae are still alive and active (fig. 4). The accumulation of these clots in appendages blocks the blood circulation, producing a gangrenous condition which

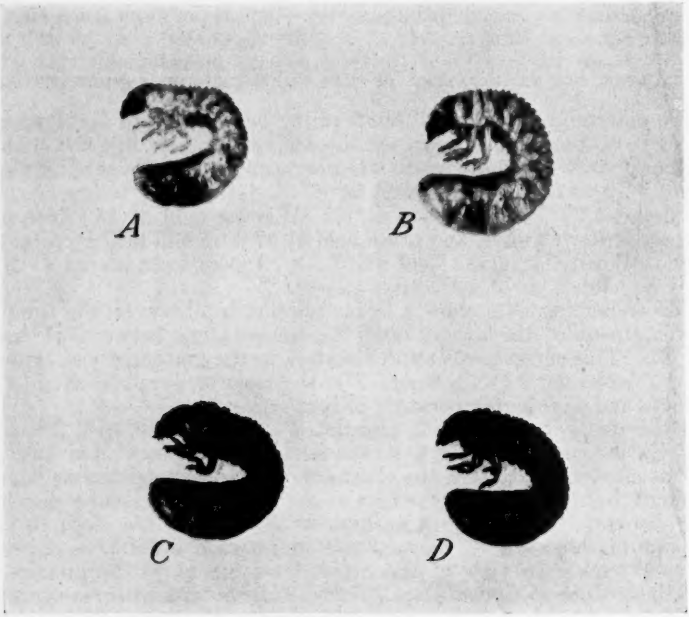

FIGURE 4.—Photograph of living larvae showing darkening of the overwintering type B milky-diseased larvae due to clot formation: *A*, Normal larva; B, overwintering diseased larva with clotting still quite limited; *C*, *D*, overwintering diseased larvae showing extensive clot formation (note the darkening of the legs caused by massing of the spores). × about 2.

causes the affected parts to blacken. Death of such larvae is probably the result of gangrene.

When healthy larvae injected with blood of the brown diseased larvae develop the disease, they show the milky-white condition rather than the brown coloration of the larvae used as inocula.

MICROSCOPIC APPEARANCE

The blood of diseased larvae shows the presence of large numbers of spindle-shaped spore-bearing rods and nonmotile vegetative rods (fig. 5). In blood of overwintering larvae the rods are nonrefractile shadow cells, and many of the spore-bearing rods show thickened and darkened membranes, which appear to be encysted by precipitation of blood about the spores. In addition, a large number of irregular opaque bodies are observed, which are blood clots.

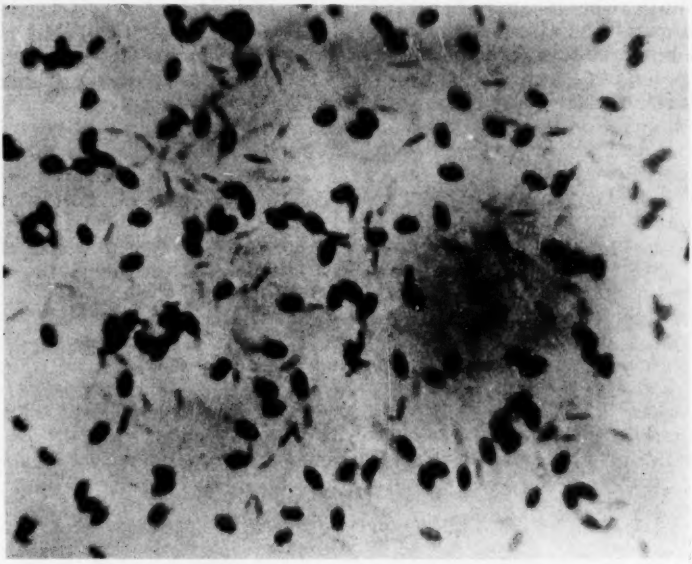

FIGURE 5.—Photomicrograph of blood of a larva with type B milky disease. Note the deeply stained spores and slender rod forms. × about 1,200.

DEMONSTRATION OF THE CAUSAL RELATIONSHIP

The disease is readily produced in healthy larvae by the injection of either heated or unheated suspensions of the blood of diseased larvae. Injection of either spores or vegetative rods produces the same sequence of events—namely, the appearance, first, of a large number of vegetative rods, followed by the typical spindle-shaped spore-bearing forms and the milky-white coloration of the larvae.

DESCRIPTION OF THE CAUSAL ORGANISM

MORPHOLOGY AND STAINING REACTIONS

The morphology and staining reactions of the vegetative form of the type B milky-disease organism are similar to those of the type A organism. The spore-bearing forms, however, although having considerable resemblance, are easily differentiated. The refractile body, so prominent in the type A sporangium, is absent in this form, and the sporangium is more decidedly spindle-shaped. The morphological differences apparent in the living spores are just as pronounced in fixed and stained spores. In the type B organism the spore-bearing rods take up crystal violet strongly and evenly, and the stained sporangium has a distinct lemon shape (fig. 6).

The dimensions of the vegetative rod are about 1.0 by 5.0 microns in the living state, and 0.5 by 4.0 microns when fixed by Schaudinn's solution and stained with crystal violet. The dimensions of the endospore are 0.9 by 1.8 microns, and of the sporangium 1.4 by 3.9 microns. When the sporangium is fixed and stained, its apparent dimensions are 1.9 by 2.8 microns.

270959—40——5

Both rods and spore-bearing forms are stained by Hucker's modification of the Gram stain. The spores retain a deep red, and the sporangia are decolorized when stained by the Dorner method.

Attempts at artificial culture and isolation of the causal organism have thus far been unsuccessful.

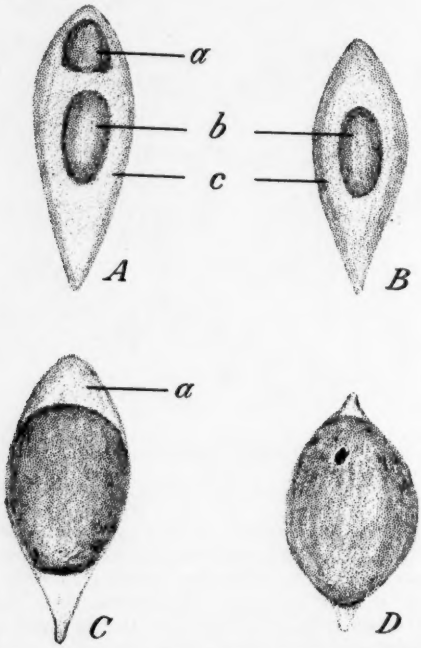

FIGURE 6.—Drawings illustrating the major morphological differences between the type A and type B spores: *A*, Type A, and *B*, type B spore unstained: *a*, Refractile body; *b*, endospore; *c*, sporangium. Note the absence of a refractile body and greater symmetry in *B*. *C*, Type A spore stained with crystal violet: *a*, Position of unstained refractile body. Note the lack of uniformity in staining. *D*, Type B spore stained with crystal violet. Note the uniform staining and lemon shape. × about 10,000.

RESISTANCE OF THE SPORES

The spores are resistant to heat, withstanding at least 85° C. for 10 minutes when heated in physiological saline suspensions. They are also resistant to desiccation, producing the disease upon injection into the healthy larvae after drying in thin blood films on glass slides for as long as 42 months.

DEVELOPMENT OF THE ORGANISM WITHIN THE HOST

Larvae were inoculated with 2 million spores by injection with spore suspensions of the causal organism, and held at 30° C. As with the type A disease, the organism is largely a blood parasite, although other tissues may be attacked. Periodic examination of

the blood showed the following changes in the organism: For 2 days after inoculation a gradual reduction in the number of spores was observed. On the third day vegetative rods appeared in considerable numbers, most of which were present in pairs. Adjoining ends of paired cells were truncate, indicating that the division probably occurred by plate formation rather than by constriction. The number of rods increased with time, and swelling of the rods began on the fifth day. At that time the presence of vacuoles was also noted in a few cells. On the sixth, seventh, and eighth days there was a growing preponderance of swollen sporulating rods, until at the ninth day they were present in sufficient numbers to give the first external symptoms of the disease.

At 30° C. development of the causal organism seems to be retarded, and the number of spores per larva seldom exceeds 1 to 2 billion even after 2 weeks at this temperature. At temperatures lower than 30° an increase in the number of spores continues after visible symptoms are observed, and after 2 to 3 weeks the typical milky-white condition of field-collected diseased larvae is reached. At this time between 5 and 10 billion spores per larva are present. Mature third instars, inoculated by injection, frequently had pupated before the disease caused their death.

EFFECT OF TEMPERATURE ON DEVELOPMENT OF THE DISEASE

When larvae were inoculated by injection and held at different temperatures, both the time and the extent of development of the disease were different. As mentioned above, although the time of development of the first external symptoms is the same (9 days) at 30° and 25° C., the extent of development is less at the higher temperature. At 22° external symptoms first appeared after 10 days, and at 15.5° after 19 days; at 12° there was no development after 63 days, and spores were in evidence but no rods.

The absolute maximum and minimum temperatures for development have not been determined for the type B organism, but the observations between 12° and 30° C. indicate that 30° is close to the maximum temperature, since the disease develops less than at lower temperatures, and that the minimum temperature is between 12° and 16°, probably closer to the latter. It seems, therefore, that the optimum and maximum temperatures for development of the type B disease are lower than those for the type A disease, and the minimum temperature for development is very nearly the same for both diseases. Type B disease thus has a considerably smaller temperature range than type A disease.

SUMMARY

Two new spore-forming bacteria are described. These organisms are the causal agents of types A and B milky disease of the larvae of the Japanese beetle (*Popillia japonica* Newm.).

The type A milky disease organism is a nonmotile Gram-positive rod measuring about 0.9 by 5.2 microns. The rods become swollen at sporulation, assuming first a spindle and then a pyriform shape. The spores are cylindrical and measure about 0.9 by 1.8 microns and are located centrally in the cell. In the broader pole of the cell

is found a refractile body, which is about half the size of the spore and possesses staining reactions similar to those of the spore. The temperature range of development seems to be from 16° to 36° C. The spores are found mainly in the blood of the larvae, reaching numbers as high as 20 billion in a single insect.

The type B organism is similar in appearance to the type A organism in the vegetative stages, but is readily distinguished morphologically after sporulation. The refractile body so prominent in the type A spore is lacking in the type B organism, and the spore-bearing rods are more nearly spindle-shaped. The temperature range of development seems to be somewhat narrower than for the type A organism, although the minimum temperature for development is the same (16° C.).

Both organisms produce a similar disease condition in the larvae of the Japanese beetle, so that upon gross examination the two conditions are usually indistinguishable.

Only the type A organism has thus far been cultured on artificial media.

The author proposes the name *Bacillus popilliae*, n. sp., family Bacillaceae, for the species causing the type A milky disease and *Bacillus lentimorbus*, n. sp., for the species causing the type B disease.

COLOR MARKINGS IN RHODE ISLAND RED CHICKS AS RELATED TO SEX AND ADULT COLOR [1]

By F. A. HAYS

Research professor, Massachusetts Agricultural Experiment Station

INTRODUCTION

Rhode Island Red chicks have long been known to vary widely in the color of their down. Warren [2] made a study of the inheritance of down color in Rhode Island Red chicks and its relation to adult plumage color and pointed out important variations in down color. Although he was not concerned with possible relations between down color and sex, he noted that striped chicks were preponderantly females.

Byerly and Quinn [3] made a study of down color in Rhode Island Red chicks in relation to sex. They studied 1,102 chicks and well-developed embryos from production-bred stock and 663 chicks from a standard-bred flock. They classified chicks for black pigment spots on the head or stripes on the back, but they made no reference to brown pigmented areas on head or back. Their data showed that 47.5 percent of the chicks examined were spotted or striped and that 84.9 percent of the spotted or striped chicks were females and 77.8 percent of the nonspotted were males. Of the striped chicks 93.6 percent were females. In standard-bred chicks 42.1 percent were either spotted or striped. Byerly and Quinn concluded that spotting and striping are widely distributed in Rhode Island Red chicks.

Quinn and Byerly [4] made a further study of spotting and striping in Rhode Island Red and New Hampshire chicks exhibited at the Northeastern Poultry Producers Exposition in 1936. These chicks represented groups selected for uniformity in down color. Of a total of 650 chicks from 26 different entries, 24.9 percent of the Rhode Island Reds and 13.3 percent of the New Hampshires showed some melanic pigment. The sex was determined on five lots of chicks from each breed and in this composite sample only 19.4 percent showed any melanic pigment. A sex ratio of 142 males to 100 females was observed. The percentage of females in the spotted chicks was 78.8. New Hampshire chicks were freer from melanic pigment than Rhode Island Reds both in selected and unselected samples.

EXPERIMENTAL METHODS

Color markings were recorded for 8,713 Rhode Island Red chicks as they were taken from the incubator in 1937, 1938, and 1939. Most of these chicks were bred for high fecundity, but a small number bred for exhibition quality were included each year. Distinction was

[1] Received for publication March 18, 1940. Contribution No. 368 of the Massachusetts Agricultural Experiment Station.

[2] WARREN, D. C. THE INHERITANCE OF RHODE ISLAND RED CHICK DOWN-COLOR VARIATIONS AND THEIR RELATION TO COLOR VARIATIONS IN ADULT PLUMAGE. Jour. Agr. Res. 39: 781–794, illus. 1929.

[3] BYERLY, T. C., and QUINN, J. P. SEXUAL DIMORPHISM IN SINGLE COMB RHODE ISLAND RED DOWN COLOR. Jour. Hered. 27: 319–322, illus. 1936.

[4] QUINN, J. P., and BYERLY, T. C. SPOTTING AND STRIPING IN EXHIBITION CLASSES OF RHODE ISLAND RED AND NEW HAMPSHIRE BABY CHICKS. Poultry Sci. 16: 422–425. 1937.

made between brown and black pigment areas, and the location of the pigmented area was indicated. No attempt was made to breed for or against spotting and striping so that the data actually represent an unselected population of pedigreed chicks. Adult color was taken at 6 months of age on part of the birds and the color classes used represent the general surface color.

DOWN COLOR OF CHICKS WHEN HATCHED

The down color of the chicks according to sex is recorded in table 1. Table 1 shows that in the male chicks 89.84 percent had no color markings; about 10 percent had either black or brown spots or stripes on the head; about 0.2 percent had black on the neck, 0.09 percent had brown on the neck; and 0.38 percent had black on the back as compared with 0.16 percent with brown on the back.

Among the female chicks 55.86 percent had solid down color and 44.14 percent had some type of spotting or striping as compared with about 10 percent of marked chicks among the males. In the total population of 8,713 chicks the probability of any one chick being a marked female was only 0.2159 and the probability of a marked chick being female was 0.806. The data show further that female chicks are much more likely to have pigment stripes on neck and back, as Warren [5] has pointed out.

TABLE 1.—*Color markings of 8,713 male and female chicks when hatched*

Color marking	Males		Females	
	Number	*Percent*	*Number*	*Percent*
None	3,997	89.84	2,382	55.86
Black head	198	4.45	1,175	27.56
Brown head	250	5.62	703	16.49
Black neck	9	.20	55	1.29
Brown neck	4	.09	19	.45
Black back	17	.38	106	2.49
Brown back	7	.16	56	1.31
Chicks observed	[1] 4,449		[1] 4,264	

[1] Several chicks appear more than once because pigmentation occurred in more than 1 area.

DISTRIBUTION OF PIGMENT IN BLACK AND BROWN PIGMENTED CHICKS

A comparison of black and brown pigmented chicks of the same sex is shown in table 2 for the 2,325 pigmented chicks.

TABLE 2.—*The relation of sex to distribution of color markings in black and brown pigmented chicks* [1]

Body part	Black pigmentation				Brown pigmentation			
	Male		Female		Male		Female	
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Head only	176	88.44	1,024	87.22	242	96.03	633	90.43
Neck only	0	0	0	0	1	.40	0	0
Back only	2	1.01	3	.26	1	.40	1	.14
Head and neck	6	3.02	47	4.00	3	1.19	17	2.43
Head and back	13	6.53	93	7.92	5	1.98	47	6.71
Head, neck, and back	2	1.01	7	.60	0	0	2	.29
Total	199		1,174		252		700	

[1] 9 chicks recorded as having black in one part of the body and brown in another are omitted

[2] See footnote 2.

When black pigment appeared in the individual there was no sex difference in its distribution. About 88 percent of males and 87 percent of females exhibited their black pigment on the head. Head and back showing black pigment in the shape of a head spot and a stripe on the back was next in importance and equally prevalent in the males and females that carried down pigmentation. Black pigment occurring as a stripe from the head along the neck was of considerable importance and probably about as frequent in males as in females in pigmented individuals. Black pigment did not appear on the neck alone; it did sometimes appear on the back alone. About 1 percent of black-pigmented chicks showed the black on head, neck, and back simultaneously.

Brown head spots were more prevalent in males than were black head spots. Brown on head and back was three times as prevalent in females as in males. Brown stripe on head and neck was more prevalent in the females. Brown pigment seldom appeared on the neck or back alone and very seldom on head, neck, and back simultaneously.

The data in table 2 indicate that black pigment spots and stripes were more common in the population studied than were brown pigmented areas. There is further evidence that some sex differences may occur in the distribution of brown pigment.

DISTRIBUTION OF COLOR MARKINGS OF CHICKS IN THE TOTAL POPULATION

A study was next made of the distribution of black and brown pigment without regard to sex in those chicks that carried pigmented spots or stripes at hatching. Table 3 records the combined data on 2,325 chicks.

TABLE 3.—*Distribution of color markings of 2,325 newly hatched black and brown pigmented chicks of both sexes* [1]

Body part	Black pigmentation			Brown pigmentation		
	Chicks	Proportion of all chicks	Proportion of males	Chicks	Proportion of all chicks	Proportion of males
	Number	*Percent*	*Percent*	*Number*	*Percent*	*Percent*
Head only	1,200	13.77	14.67	875	10.04	27.66
Neck only	0	0		1	.01	100.00
Back only	5	.06	40.00	2	.02	50.00
Head and neck	53	.61	11.32	20	.23	15.00
Head and back	106	1.22	12.26	52	.60	9.62
Head, neck, and back	9	.10	22.22	2	.02	0
Total	1,373	15.76	14.49	952	10.93	26.47

[1] 9 chicks recorded as having black in one part of the body and brown in another are omitted.

Of the chicks that showed black on the head only 14.67 percent were males, indicating that about 85 percent of the chicks with black pigment on the head were females, an observation which agrees closely with the findings of Byerly and Quinn.[6] When the head spot was brown 27.66 percent of the chicks were males. The appearance of black spots or stripes on head, neck, or back, or various combinations of these areas, showed about the same relation to sex. When the

[6] See footnote 3.

pigment was brown it generally appeared on the head and was somewhat less closely associated with sex. Chicks marked with black represented 15.76 percent of all chicks, and those marked with brown represented 10.93 percent of all chicks. There were, therefore, 26.69 percent of all chicks that showed some color markings. From the total of 8,713 chicks there were only 26.69 percent whose down pigmentation could be used as a guide in separating sex. Considering the marked chicks only, 14.49 percent of those marked with black were males and 26.47 percent of those marked with brown were males. When all chicks marked with black were combined with all marked with brown, 19.40 percent were found to be males and 80.60 percent females. As a practical means of separating sexes, down spots and stripes are of little value.

RELATION OF DOWN COLOR MARKINGS TO ADULT COLOR

An attempt was made to discover any relations that might exist between black and brown down color markings and general adult plumage color. For this purpose it was considered advisable to classify the birds at 6 months of age for general color of surface plumage rather than for color in different body regions. Adult plumage color was grouped into 5 general classes as shown in table 4 and male and females were considered separately and combined. Adult colors were recorded during the last 2 years of the study on 1,295 males and on 2,493 females. The data are summarized in table 4.

TABLE 4.—*Color markings of chicks at hatching as related to adult plumage color and to sex, 1938 and 1939*

Sex and adult color	Adults which, as chicks, were—						Total	
	Nonmarked		Black-marked		Brown-marked			
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Males:								
Very light red	21	1.73	0	0	0	0	21	1.62
Light red	684	56.39	20	37.04	13	46.43	717	55.37
Medium red	341	28.11	17	31.48	11	39.29	369	28.49
Dark or standard red	125	10.31	16	29.63	3	10.71	144	11.12
Black mottled	42	3.46	1	1.85	1	3.57	44	3.40
Total	1,213		54		28		1,295	
Females:								
Very light red	76	5.13	34	4.70	25	8.65	135	5.42
Light red	784	52.94	312	43.15	153	52.94	1,249	50.10
Medium red	451	30.45	233	32.23	92	31.83	776	31.13
Dark or standard red	122	8.24	128	17.70	9	3.11	259	10.39
Black mottled	48	3.24	16	2.21	10	3.46	74	2.97
Total	1,481		723		289		2,493	
Both sexes:								
Very light red	97	3.60	34	4.38	25	7.89	156	4.12
Light red	1,468	54.49	332	42.73	166	52.37	1,966	51.90
Medium red	792	29.40	250	32.18	103	32.49	1,145	30.23
Dark or standard red	247	9.17	144	18.53	12	3.79	403	10.64
Black mottled	90	3.34	17	2.19	11	3.47	118	3.12
Total	2,694		777		317		3,788	

Of the 1,213 males that had no pigment spots when hatched, 56.39 percent were classed as light red in adult plumage and 28.11

percent as medium red. Slightly over 10 percent approached the dark or standard red in adult color, and 1.73 percent were very light red. Black mottling appeared in 3.46 percent of the nonmarked males when they developed their adult plumage.

The small group of male chicks that had some black markings exhibited a somewhat darker adult plumage than was observed in nonmarked chicks. About 61 percent of these black-marked chicks showed medium or dark adult plumage color. Only 37 percent of these males marked with black were recorded as light red in adult plumage and none as very light red. Black mottling was less common also in the black-marked males.

A very small number of male chicks had brown spots or stripes. There was some evidence that brown spotting or striping was associated with light adult plumage color. The total male population showed light red and medium red as the prevailing adult color.

Female chicks that were not marked showed a higher percentage of very light red individuals than the males and a somewhat smaller percentage of dark red. The incidence of black mottling was almost the same in both sexes. The data suggest in general that the prevailing shade of adult color was slightly lighter in females than in males on a relative basis.

Black-marked female chicks developed a little darker adult color than did the nonmarked females. The percentage of dark-red birds among the black-marked females was twice as large as that among the nonmarked females. The percentage of light-red individuals was smaller in the black-marked group and black mottling was somewhat less frequent.

Brown-marked female chicks tended to run a little lighter in adult plumage than nonmarked or black-marked chicks. There was a very small percentage with dark plumage color. Black mottling was equally frequent in brown-marked and nonmarked chicks. Adult males were slightly darker than females. In both sexes the appearance of black pigment in down appears to be associated with darker shades of general plumage color in adults. Differences observed in the adult color of black-marked chicks as compared with nonmarked or brown-marked chicks are not outstanding but do appear to exist.

In the total population light-red adult color prevails in the three kinds of chicks. There again appears to be an advantage from the standpoint of darker adult color and freedom from mottling in selecting chicks with black pigmented areas. In a flock bred for high fecundity where the general plumage color tends to run light, something may be gained in plumage color by selecting chicks that show black pigment spots or stripes.

SUMMARY AND CONCLUSION

A study of 8,713 chicks largely bred for egg production was made to determine the relation of down pigmentation to sex and to adult color.

Only about 10 percent of male chicks showed any color markings in the down as compared with about 44 percent of female chicks that showed spotting or striping. In the total population only 26.76 percent of the chicks had spots or stripes in the down.

In the male chicks 4.47 percent had black pigmented areas and 5.66 percent had brown pigmented areas; in the females 27.53 percent had black pigmented areas and 16.62 percent had brown.

The prevailing type of pigment area was a spot on the head. In the population with pigmented down, black was confined to the head as often in males as in females. Brown pigment spots were slightly more common in male chicks in the population showing pigment spots. When chicks were striped the color of pigment was more likely to be black.

Considering only the marked population, about 15 percent of the chicks with black pigment were males and about 26 percent with brown pigment were males.

Adult color in males averaged slightly lighter than in females.

Chicks of both sexes that carried some black pigmented areas developed a slightly darker adult plumage color and fewer mottled individuals than chicks with solid down color or with brown pigmented areas.

Color markings in down has no commercial value as an indicator of sex in the stock studied.

THE SIGNIFICANCE OF THE "CEASED" REACTOR TO BANG'S DISEASE [1]

By B. A. BEACH, *professor of veterinary science, Wisconsin Agricultural Experiment Station;* M. R. IRWIN, *cooperative agent, Bureau of Animal Industry, and professor of genetics, Wisconsin Agricultural Experiment Station;* and L. C. FERGUSON, *cooperative agent, Bureau of Animal Industry, and instructor in veterinary science and genetics, Wisconsin Agricultural Experiment Station.*

INTRODUCTION

Are "ceased" reactors (i. e., those animals that have lost their agglutinin titer to *Brucella abortus* following infection) a menace to the herds in which they are kept? This question presents an important practical problem to the owners of the many herds infected with the organism producing Bang's disease. In the Federal-State Bang's disease program for eliminating reactors (i. e., those whose sera agglutinate the causative organism at an appreciable titer) from the participating herds, the problem is also of importance since in many of these herds there undoubtedly are some animals that were once reactors, but cannot now be surely recognized as such. An investigation was made to find an answer to the question about ceased reactors, and the results are reported in the present paper.

MATERIALS AND METHODS

The ceased reactors were observed in three different herds of cows, Nos. 1, 2, and 3, each of which had been artificially exposed to *Brucella abortus*.

Herd 1 was assembled in 1926, and originally consisted of 44 grade and purebred Holstein calves. The pregnant animals, 37 individuals out of the original 44, were exposed to *Brucella abortus* in 1928, with results as previously described by Hart and his coworkers.[2]

After observations had been made on these individuals over a period of 2 years following their exposure, 14 were chosen as ceased reactors. (All the other animals of this herd were slaughtered and examined for the presence of *Brucella abortus*.) However, the serum of cow No. 40, cited in table 1, had reacted only slightly with *Brucella abortus* at a dilution of 1:200, that of Nos. 4, 5, and 31, had reacted slightly at a dilution of 1:100, while that of No. 18 had never shown a definite agglutination with this organism.

The object of the writers in including these five animals in the experiment and in presenting the data in the table, along with those of herd 2 whose sera gave only a low titer, or none, in agglutination of the pathogen, was because of the possibility that such animals might be carriers of the organism. (For example, after exposure of the cows of herd 3, the serum of one animal, 20C, never showed definite agglutina-

[1] Received for publication December 4, 1939. From Paper No. 251, the Department of Veterinary Science and the Department of Genetics, Wisconsin Agricultural Experiment Station; investigation carried out in cooperation with the Bureau of Animal Industry, U. S. Department of Agriculture.

[2] HART, E. B., HADLEY, F. B., and HUMPHREY, G. C. THE RELATION OF NUTRITION TO CONTAGIOUS CATTLE ABORTION. Wis. Agr. Expt. Sta. Res. Bul. 112, 45 pp., illus. 1932.

tion of the pathogen. Furthermore, she produced a normal calf at full term, yet guinea-pigs inoculated with her uterine fluid gave evidence of the presence of the organism.) It is a point of interest that the organism was recovered from 9 of these 14 cows, following examination of their aborted fetuses, fetal membranes, and colostrum by the usual method of guinea-pig inoculation.

TABLE 1.—*The extremes of the titer of agglutinins and the dates on which these first appeared, for 28 cows in 3 herds whose serum wholly or nearly ceased to react with Brucella abortus, after infection was artificially induced by different methods*

HERD 1, EXPOSURE BY FEEDING [1] AND CONTACT WITH NATURALLY INFECTED COWS, 1928

Cow No.	Method of exposure	Serum reaction		
		Highest titer and time first observed		Time when titer disappeared, or nearly so
		Titer [2]	After number of months shown	After number of months shown
4	Several feedings, September to November	[3] S–1:100	2	3
5		S–1:100	4	6
8		1:200	3	6
12		1:200	1	24
17		1:200	4	6
18		[4]		
28		1:100	1	24
31		S–1:100	3	6
33		1:200	3	24
35		1:200	3	24
36	Contact	1:100	5	7
38	Several feedings, September to November	1:200	3	6
40		S–1:200	4	5
44		1:100	1	6

HERD 2, EXPOSURE BY INSTILLING A VIRULENT CULTURE INTO THE EYE, AND CONTACT WITH A NATURALLY INFECTED COW, 1933 [5]

[6] 73A	Eye installation and natural exposure	[4]		
32A		1:100	3	16
58A		1:50	2	5
63A		1:25	1	5
9B		1:25	3	5
10B		1:50	2	4
15B		1:100	2	5

HERD 3, EXPOSURE BY FEEDING, EXCEPT AS NOTED

3C	Feeding of approximately 142,109 organisms	1:400	4	19
12C		1:200	3	21
20C		[4]		
24C		1:200	1	9
33D		1:400	4	24
4H		1:100	2	3
38D	Instillation into eye	1:200	2	8

[1] Stomach contents from aborted fetuses and cultures were used.
[2] Unless otherwise stated, the titers represent complete agglutination of the organism at the stated dilutions of the serum.
[3] S indicates a slight reaction at the dilutions shown.
[4] Either always negative or only weak agglutination.
[5] The cow that developed mastitis after being put into herd 3, is not included.
[6] This cow had previously passed through an out break of Bang's disease.

Herd 2 was assembled in August 1931, and consisted of the 14 ceased reactors from herd 1 and 22 cows, mostly virgin heifers (normals). Within the knowledge of the writers, none of these 22 individuals had

had any previous contact with *Brucella abortus*, except 1 cow (No. 23, A table 1) that had passed through an outbreak of *Br. abortus* some years previously. The serum of 1 of these 22 cows showed the presence of agglutinins for the organism about 2 months after the herd was brought together, and this cow was immediately removed.

All the other animals, both ceased reactors and normals, calved once, a few twice, in the period between the assembly of the herd and the artificial exposure. Five animals, one ceased reactor and four of the normal group, were for various reasons removed from the herd before the artificial exposure to *Brucella abortus*. Three normal, pregnant cows were added to the herd approximately 3 months before the exposure. All animals which had been diagnosed as pregnant were artificially exposed by way of the eye in September 1933. Those which were exposed for the first time and whose sera gradually lost their agglutinins for *Br. abortus*, eight in all, were considered to be ceased reactors. The remaining animals of the herd were slaughtered, and examination was made of each for the presence of the infecting organism.

With the 8 ceased reactors from herd 2 (table 1) as a nucleus, a repetition of the experiment was begun in August 1934. Twenty-eight virgin heifers were purchased from herds free of Bang's disease and placed with the 8 ceased reactors, these together constituting herd 3. One of the 8 individuals developed acute mastitis early in the experiment and was removed; therefore, only 7 individuals are listed in the table. During the course of the experiment, 5 normal cows were added to the herd in April 1936, and 11 were removed at various times and for different reasons. Each of these 16 individuals, however, calved at least once while in the herd, thus having had ample opportunity for contact with the 7 ceased reactors. Each of the other cows calved at least once, the majority twice, and a few three times, before the artificial exposure. Two abortions were observed among the normal animals. These, however, presumably were not caused by *Brucella abortus*, since agglutinins for the organism were not found in the serum of either of the two cows, and negative results were obtained by guinea pig inoculation and culture of the placenta and uterine fluid from each.

The individuals that were diagnosed as pregnant were artificially exposed to *Brucella abortus* in May 1937. The majority of those undergoing their first contact with the pathogen were fed a massive dose of the organism, while the ceased reactors were exposed by instilling a given amount of the culture into the eye.

In both of these herds (Nos. 2 and 3), the ceased reactors and normal cows were placed together in the same barn, barnyard, and pasture. While in the stable, the normals were so placed as to give as much contact as possible with the ceased reactors. As stated, a few animals were removed from these herds during the experiment, usually because of failure to become pregnant. Any replacements that were made are mentioned only if they had contact while pregnant with the ceased reactors.

Tests to determine the agglutinin titer of the serum of all animals in herds 2 and 3 were usually made at monthly, often at weekly, intervals. Both plate and tube methods of determining the titer of the serum were used, with reasonably close agreement in the titer as

found by the two methods. Independent readings of different samples of sera were often made by different workers.

Throughout the course of these experiments (i. e., beginning with the assembly of herd 2 in 1931), in order to determine whether the ceased reactors were carriers of the organism, the colostrum and either the placenta or the uterine fluid, or both, were tested at each parturition for the presence of *Brucella abortus* by the usual method of guinea-pig inoculation.

RESULTS

HERD 2

A summary of observations on the ceased reactors in herd 2 has already been reported,[3] and only a brief mention of certain of the findings will be made here. The titer for *Brucella abortus* of the sera of the 14 ceased reactors of this herd was determined monthly, or oftener, during the period from the beginning of the experiment in 1931 to the artificial exposure of all the pregnant animals of the herd in 1933. Eleven of the ceased reactors were negative, or at most suspicious, in their reaction to the organism during this period. The sera of 3 of these individuals at times gave a low reaction. Further, injection into guinea pigs of the colostrum from each quarter of the udder and uterine fluid from each of these 14 cows failed to reveal *Br. abortus*, except in one instance, when from 1 cow, at 1 freshening only, *Br. abortus* was recovered from the colostrum from 1 quarter of the udder.

During these tests it was noted that the serum from the colostrum from each of these cows showed the presence of antibodies (agglutinins) for the organism at a dilution of 1:400, or higher. This titer, however, disappeared after a few milkings. This finding suggests that there had been a concentration of antibodies in the udders during the nonlactating period.

Shortly after the assembly of herd 2, the serum of one of the normal individuals showed a low titer (1:100) for the organism. This animal was immediately removed from the herd and kept in isolation until after calving. The titer of her serum receded slightly, and the organism was not recovered at calving (normal), following the usual guinea pig injections, or from the body organs when the cow was slaughtered shortly after calving. Except for this one individual, there was no indication of an increase in titer of the serum of the individuals of this group which presumably were noninfected when introduced into the herd.

The exposure of the cows in herd 2 was accomplished by instilling virulent cultures into the eye, and also by introducing an infected cow into the herd. There were 20 cows pregnant at that time that had not previously been exposed to the organism. Following the exposure, the serum of 17 of these showed an agglutinin titer for *Brucella abortus* of 1:50 or higher. The serum of 5 of these 17 cows gradually lost agglutinins for the pathogen. These 5, together with 3 others (9B, 23A, and 63A in table 1) that never showed more than a suspicious reaction, if any, made up the 8 ceased reactors which later were placed

[3] BEACH, B. A., and HUMPHREY, G. C. THE PRESENCE OF BR. ABORTUS IN THE UTERINE FLUID AND IN THE MILK AND OF AGGLUTININS IN THE BLOOD SERA OF SO-CALLED CEASED REACTOR COWS. Vet. Med. 30: 8-10. 1935.

in herd 3. It will be noted, therefore, that 5 out of 17 reactors, or approximately 29 percent, ceased to react. These 5 cows had normal calves after exposure. One of the cows which had given a suspicious agglutination reaction aborted; the other 2 calved normally.

HERD 3

Herd 3, as previously explained, was started on the experiment in August 1934. From the time the herd was assembled to the time that the pregnant individuals were artificially exposed in the late spring of 1937, all the animals remained negative or nearly so, in their agglutination reaction. Only occasionally the serum of one or more of the seven ceased reactors showed a low agglutinin titer.

In this herd, as in herd 2, the colostrum from each quarter of the udder and either the placenta or the uterine fluid, or both, from each animal, whether a ceased reactor or normal, at the time of calving were injected into guinea pigs. In no instance was there any indication of the presence of *Brucella abortus* in the injected material.

At the time that the animals of herd 3 were infected, there were 24 cows that had not previously been exposed to the organism. Two of these were exposed by instilling 3 drops of a virulent culture into the eye of each; each of the remaining 22 individuals was fed 20 cc. of a heavy suspension of the same culture. Of these 24 cows, 6, or 25 percent, became ceased reactors within a period of 24 months after the artificial exposure. One cow (20C in table 1), although proved to have been infected because of the undoubted infection of the guinea pigs injected with the uterine fluid at the calving following the artificial infection, never gave any evidence of a rise in agglutinins following the infection.

DISCUSSION

From the information given in table 1, it will be seen that it was not possible to predict, by the height of the agglutinin titer, whether or not an individual would become a ceased reactor. A summation of the individuals of table 1 which in the three different herds became ceased reactors reveals that, for those whose sera showed complete agglutination at the maximum titer given, there were two with a titer of 1: 400, nine with 1: 200, six with 1: 100, and four with less than 1: 100. Also, there were four animals in herd 1 whose highest titer was 1: 200 or less, as evidenced by a slight trace of agglutination at the titer shown. Furthermore, there were three animals in these different groups which failed to react after the artificial exposure. The animals whose sera never reacted at the prescribed dilution for reactors cannot strictly be classed with the ceased reactors, although in these tests they were included because they might have been carriers of the organism. One such individual (20C in herd 3) definitely was a carrier and therefore a probable spreader. One of the three cows (23A in herd 2) whose sera had never agglutinated the organism, had been in a herd which several years previously had suffered an outbreak of Bang's disease, and thus may have been a ceased reactor when introduced into herd 2.

There can be no doubt of the adequacy of the artificial infection in each of these different herds, since in both herds 2 and 3 more than

half of the cows undergoing their first exposure to *Brucella abortus* aborted their fetuses. In fact, the proportion of aborting cows was higher in both herds 2 and 3 than in herd 1. The details of these infections, and of the response of the ceased reactor cows to second exposure, will be reported elsewhere.

SUMMARY AND CONCLUSIONS

Twenty-one so-called "ceased" reactor cows in 2 herds (14 in 1, 6 in the other) were allowed to commingle with a total of 54 cows from Bang-negative herds through either 1 or 2 gestation periods for each of the normal cows. With the exception of a transitory infection in one-quarter of the udder of 1 of the ceased reactors, *Brucella abortus* was not demonstrated in any of these cows by culture or guinea pig injection at the time of calving. Moreover, none of the 54 normal cows showed evidence of having been infected by any of the ceased reactors, as evidenced by the lack of agglutinins in their sera for *Br. abortus*, except 1 individual whose serum showed a low titer (1: 100) for the organism. This one reactor was in the same herd as the cow showing the transitory infection cited. The writers were unable to demonstrate the organism in this cow either at the time of calving or after slaughter.

The results of these experiments indicate that it is relatively safe to allow ceased reactors to Bang's disease to mingle with noninfected stock. It cannot definitely be said that a ceased reactor cannot be a carrier of *Brucella abortus*, but on the basis of these experiments, it can be stated that the probability is undoubtedly small.

U. S. GOVERNMENT PRINTING OFFICE: 1940